# Metabolic and hydration responses associated with the ingestion of a sport drink with caffeine and increased concentration of carbohydrate.

Ruiz, Carlos Penas

COLLEGE OF ENGINEERING
SWANSEA UNIVERSITY

# Metabolic and Hydration Responses Associated with the Ingestion of a Sport Drink with Caffeine and Increased Concentration of Carbohydrate

Swansea University
Prifysgol Abertawe

**Carlos Peñas Ruiz**

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Ann Arbor, MI 48106 – 1346

## Abstract

It is widely accepted that carbohydrate ingestion previous and during exercise improves exercise performance. Additionally, the ingestion of carbohydrates can improve skills performance in team sports (e.g. soccer, rugby, hockey). Carbohydrate drinks are widely utilised in sports settings. However, the optimal composition for this type of drinks has not been clearly established. The effects on metabolic and hydration status of ingesting a sport drink with a higher carbohydrate load (>8%) and caffeine are not well understood.

Fourteen male recreational soccer players completed a soccer match simulation protocol (SMS) that replicates the physiological responses and activity patterns of soccer match-play. Participants completed three exercise trials, ingesting a 6% carbohydrate drink with a carbohydrate gel (CHO6), a 10% caffeinated-carbohydrate drink with carbohydrate gel (CHO10) and a flavoured placebo drink with a placebo gel (PLA) in a double-blind and randomised order. Blood glucose, blood lactate and rate of perceived exertion were assessed every fifteen minutes during the SMS. Heart rate was continuously recorded throughout each trial using short-range telemetry. Additionally, the participants' hydration status was assessed (body mass changes, urine and plasma osmolality, plasma sodium concentration, and plasma volume changes) throughout the SMS protocol. Sprint speed was recorded as an indicator of high-intensity exercise performance.

Carbohydrate ingestion was effective in elevating blood glucose concentrations during the first half of the protocol when compared with the flavoured placebo. However, blood glucose concentrations decreased by 40%, 38%, and 16% in the CHO10, CHO6 and PLA trials, respectively, after the half-time recovery period. The ingestion of a 10% caffeinated carbohydrate drink resulted in a 2% increase in plasma osmolality at the end of the SMS protocol (pre-SMS: 292 ± 3 mosm·l$^{-1}$; post-SMS: 298 ± 3 mosm·l$^{-1}$, $P<$ 0.05). Plasma sodium concentration was elevated in the CHO6 and CHO10 trials (CHO10 = 143 ± 0.7 mosm·l$^{-1}$, CHO6 = 141 ± 0.8 mosm·l$^{-1}$, PLA = 140 ± 0.8 mosm·l$^{-1}$; $P<$ 0.05). Sprint performance was improved with the ingestion of 10% caffeinated carbohydrate drink at 45 min when compared with the CHO6 and PLA trials (CHO10 = 5.73 ± 0.08 m·s$^{-1}$, CHO6 = 5.59 ± 0.09 m·s$^{-1}$, PLA = 5.58 ± 0.11 m·s$^{-1}$; $P<$ 0.05).

In conclusion, the ingestion of a 10% carbohydrate caffeinated drink along with a carbohydrate gel was effective in elevating blood glucose concentration and improving sprint performance when compared with a placebo treatment during a soccer-specific protocol. Additionally, the ingestion of a 10% caffeinated-carbohydrate drink along with a carbohydrate gel did not critically affect hydration status in temperate conditions. However, players should be cautious when ingesting highly concentrated carbohydrate beverages in hot and humid conditions.

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## List of Abbreviations

| | |
|---|---|
| ACSM | American College of Sports Medicine |
| ADA | American Dietitians Association |
| ADH | Antidiuretic Hormone |
| ADP | Adenosine Diphosphate |
| ANOVA | Analysis of the variance |
| ATP | Adesonsine Triphosphate |
| BM | Body Mass |
| CHO | Carbohydrate |
| CHO6 | 6% Carbohydrate-Electrolyte Beverage + Active Gel |
| CHO10 | 10% Carbohydrate-Electrolyte/Caffeine Beverage + Active Gel |
| CoA | Coenzyme A |
| CV | Coefficient of variation |
| d.w. | Dry weight |
| $FADH_2$ | Flavin adenine dinucleotide |
| FIFA | Fédération Internationale de Football Association |

| | |
|---|---|
| GLUT5 | Glucose Transporter Type 5 |
| $H_2O_2$ | Hydrogen peroxide |
| $HO_{1, 2, 3}$ | Hypotheses 1, 2, 3 |
| ISAK | International Society for the Advancement of Kinanthropometry |
| $K^+$ | Potassium |
| LIST | Loughborough Intermittent Shuttle Test |
| MSFT | Multi Stage Fitness Test |
| $Na^+$ | Sodium |
| NADH | Nicotinamide Adenine Dinucleotide |
| $\eta_p^2$ | Partial Eta Squared |
| PCr | Phosphocreatine |
| $P_i$ | Inorganic Phosphorus |
| PLA | Placebo Beverage with Placebo Gel |
| Pre-SMS | Previous the Soccer Match Simulation |
| Post-SMS | After the Soccer Match Simulation |
| RPE | Rate of Perceived Exertion |
| SEM | Standard Error of the Mean |
| SGLT1 | Sodium Dependant Glucose Transporter 1 |
| SMS | Soccer Match Simulation Protocol |
| SPSS | Statistical Package for the Social Sciences |
| $\dot{V}O_{2max}$ | Maximal Oxygen Consumption |

## List of Appendices

# Chapter 1

## Introduction

## 1.0 Introduction

Soccer (association football) is commonly regarded as the most popular sport worldwide, with 300 million registered individuals regularly involved in soccer activities (Dvorak, 2011). With this level of popularity it is not surprising to find large number of scientific studies focused on the different aspects of soccer performance. Two of the most commonly investigated areas in soccer are the factors related with exercise-induced fatigue and the physical and nutritional strategies utilised to attenuate the effects of fatigue during games (Shephard, 1999). Fatigue in soccer can occur at different times during a game. Temporary fatigue has been associated with disturbed muscle ion homeostasis (Ekblom, 1986; Mohr *et al.*, 2004b; 2005). The decrease in performance observed after the half-time period has been related to muscle temperature decrease (Bangsbo, 1995; Mohr *et al.*, 2004a) and low blood glucose concentration. The most apparent fatigue is the one observed during the second half of the game. This form of fatigue has been associated with muscle and liver glycogen depletion (Krustrup *et al.*, 2006; Saltin, 1973), dehydration (Maughan and Leiper, 1994; Reilly, 1997), hyperthermia and hypernatraemia (Drust *et al.*, 2000).

Since carbohydrate has become the preferred food for soccer training and competition (William and Serratosa, 2006), numerous carbohydrate supplementation strategies have been used to enhance soccer performance. It is widely accepted that carbohydrate ingestion during exercise can prolong time to exhaustion (Nicholas *et al.*, 1995; Patterson and Gray, 2007; Foskett *et al.*, 2008), improve sprint performance (Ali *et al.*, 2007) and enhance soccer skills (Currell *et al.*, 2009; Ostojic and Mazic, 2002). The mechanisms behind the performance improvements associated with carbohydrate ingestion are not clear, but some authors have pointed to the maintenance of blood glucose homeostasis and the delay of muscle glycogen depletion as the responsible factors for these improvements (Ali *et al.*, 2007, Currell *et al.*, 2009; Nicholas *et al.*, 1995).

The ingestion of carbohydrate within the hour before exercise has been associated with a transient drop in blood glucose concentration due to an increase in circulating insulin

and an increase in glucose uptake by muscle at the onset of exercise (Coombes and Hamilton, 2000; Kuipers *et al.*, 1999). Generally, blood glucose homeostasis is maintained during soccer games (Bangsbo *et al.*, 2007). However, several studies, including pilot observations performed previous to the present study (Appendix H), revealed reductions in blood glucose concentration at the beginning of the second half of a soccer-specific exercise protocol (Russell *et al.*, 2011a) or soccer games (Krustrup *et al.*, 2006). Therefore, the aim of the present study was to investigate the effects of caffeine and carbohydrate ingestion on blood glucose response during a soccer-specific exercise protocol.

Most of the commercially available sport drinks have a concentration of carbohydrate ranging from 5 to 8% of carbohydrate, with osmolalities from 240 to 360 $mosm \cdot kg^{-1}$ (Coombes and Hamilton, 2000). The most utilised carbohydrate molecules in this type of beverages are glucose, fructose, sucrose and synthetic glucose polymers (maltodextrin). Some of these drinks use the combination of caffeine with carbohydrate to enhance the ergogenic effects of the drink.

It has been suggested that glucose oxidation rate is mainly limited by intestinal carbohydrate absorption to about 1.1 $g \cdot min^{-1}$, when carbohydrates are ingested at rates of 1.2-1.5 $g \cdot min^{-1}$ (Jentjens *et al.*, 2004). However, carbohydrate oxidation might reach peak values of 1.5-1.7 $g \cdot min^{-1}$ when a mixture of multiple transportable carbohydrate are ingested at rates of 1.5-2.4 $g \cdot min^{-1}$ during exercise (Jentjens and Jeukendrup, 2005a; Wallis *et al.*, 2005). Additionally, the ingestion of caffeine combined with carbohydrate might increase the rate of exogenous carbohydrate oxidation (Yeo *et al.*, 2005). Therefore, the ingestion of a high dose of multiple transportable carbohydrates combined with caffeine may increase the rate of carbohydrate absorption in the small intestine and facilitate the oxidation of carbohydrate, hence improving exercise performance.

The ingestion of sport drinks does not only provide individuals with an additional source of exogenous carbohydrate for muscle and brain metabolism, but maintains adequate body water and electrolytes balance during exercise. The ingestion of multiple

transportable carbohydrates at rates higher than 1.5 $g \cdot min^{-1}$ has resulted in performance improvements during prolonged continuous exercise (Maughan *et al.*, 1989; Mitchell *et al.*, 1989). This carbohydrate ingestion strategy is related with the maintenance of blood glucose homeostasis and the attenuation of endogenous carbohydrate production (Jeukendrup *et al.*, 1999). However, the ingestion of highly concentrated carbohydrate drinks have also resulted in low oxidation efficiency (63-73%), which in turn increases the accumulation of carbohydrate in the gastrointestinal tract and cause gastrointestinal discomfort (Rowlands *et al.*, 2008). Additionally, the accumulation of carbohydrate in the small intestine tends to increase the gut osmotic gradient, causing a movement of water across the intestinal wall to balance the osmotic pressure in the intestinal lumen (Evans *et al.*, 2009). Therefore, the ingestion of carbohydrate at high rates might increase the effects of exercise-induced dehydration.

Despite of the controversial effects of ingesting highly concentrated carbohydrates drinks during prolonged exercise, only a limited number of studies have investigated its effects on performance during high-intensity intermittent exercise (Murray *et al.*, 1989; Phillips *et al.*, 2011; Welsh *et al.*, 2002). To the date, no study has investigated the effects of the ingestion of carbohydrate beverages differing in the concentration and composition on hydration during intermittent exercise. Therefore, the study aimed to identify the effects of ingesting a beverage with a high dose of carbohydrate combined with caffeine on hydration status and exercise performance during soccer-specific exercise protocol.

## 1.1 Null Hypotheses

**$HO_1$**: Blood glucose concentrations will not be influenced by the ingestion of a beverage with caffeine and an increased concentration of carbohydrates (10%) compare with a typical carbohydrate beverage (6% carbohydrate).

**$HO_2$**: Hydration indices will not be influenced by the ingestion of a beverage with caffeine and an increased concentration of carbohydrates (10%) compare with a typical carbohydrate beverage (6% carbohydrate).

**$HO_3$**: Sprint performance will not be influenced by the ingestion of a beverage with caffeine and an increased concentration of carbohydrates (10%) compare with a typical carbohydrate beverage (6% carbohydrate).

# Chapter 2

## Review of the Literature

## 2.1 Science and Soccer

Soccer (association football) is commonly regarded as the most popular sport worldwide (Bangsbo, 1994), performed by men and women, children, adults, disabled and able body individuals. According to FIFA estimates, there are around 300 million registered individuals regularly involved in soccer (players, referees and coaches), with hundreds of thousands organised games played every week around the globe (Dvorak, 2011). The game popularity is increasing every year, with more spectators following the national and international competitions played around the globe. Clear evidence of the increased popularity of soccer is the FIFA World Cup, which draws the attention of millions of fans every four years (Dvorak, 2011).

The factors associated with the improvement of performance in team sports have been widely investigated to support and develop the players' performance. One of the most investigated areas in sport is the use of carbohydrate supplementation during endurance, high-intensity or intermittent exercise events. As carbohydrate has become the athletes' preferred nutrient, the production and sale of carbohydrate products (e.g. sport drinks, snack bars and gels) has turned into a lucrative and competitive industry, with numerous new products being launched to the market every year. Sports drinks are typically formulated to prevent dehydration, supply an additional source of carbohydrates, replace lost electrolytes and be palatable to facilitate the ingestion of fluids (Coombes and Hamilton, 2000). The ingestion of carbohydrate drinks has been widely tested to provide information about its effects on exercise performance and human metabolism. This review of the literature examines the characteristics of soccer performance, the forms of fatigue during soccer games and the effects of carbohydrate supplementation on exercise performance and hydration.

## 2.2 Soccer Activities

Notational and motion analysis techniques have been used to describe and quantify the physical and skill performance during soccer games. Early studies used video recording (Reilly and Thomas, 1976; Van Gool *et al.*, 1988), hand notation (Brookes *et al.*, 1974;

Ekblom, 1986) and trigonometry (Ohashi *et al.*, 1988) to describe the players' movement patterns during games. These methods were time consuming and did not always produce reliable data, due to a large influence of human error in the notational techniques (Carling *et al.*, 2008). Over the last decade, the development of computerised, semi-automatic video analysis techniques have allowed top-level soccer teams to obtain detailed match performance data just a few hours after the game (Rampinini *et al.*, 2007).

Soccer players perform a wide range of activities described as a succession of rapid multi-directional high-intensity movements, with or without possession of the ball, combined with medium and low intensity activities (Carling and Dupont, 2011). Top players cover approximately 25% of the total distance walking, 37% jogging, 20% cruising, 11% sprinting and 7% running backwards (Reilly, 1997). Although most of the exercise is performed at low intensities, players perform 2-3 second sprints every 90 seconds.

### 2.2.1 Total Distance Covered

Professional players cover approximately 10 km during soccer games (Barros *et al.*, 2007; Rampinini *et al.*, 2007; Rampinini *et al.*, 2009), with midfield players usually covering slightly more distance than players in other field positions (Bradley *et al.*, 2009; Vigne *et al.*, 2010). Several authors have indicated that the tactical approach of the team (Carling and Bloomfield, 2010; Lago *et al.*, 2009) and the importance of the competition in play (Rienzi *et al.*, 2000) might influence the total distance covered in a game. Soccer players cover less distance during the second half of the game when compared with the first half (Mohr *et al.*, 2003; Reilly and Thomas, 1976; Rienzi *et al.*, 2000; Van Gool *et al.*, 1988). The decrease of distance covered during the second half has been widely associated with the onset of fatigue (Bangsbo *et al.*, 2007; Mohr *et al*, 2003; Rampinini *et al.*, 2007).

### 2.2.2 High-intensity Activities

The overall activity pattern in soccer games indicates that exercise is mostly performed at low- or moderate-intensity; however, high-intensity activities are commonly associated with crucial actions of the game (Reilly, 1997). A study by Mohr *et al.* (2003) showed that players perform 150-250 brief high-intensity activities (sprints, short accelerations and jumps) accounting for 30% of the match-play time. Additionally, soccer players perform a sprint lasting 2-4 s approximately every 90 s (Stølen *et al.*, 2005). The distance covered at high-intensity running during games can be used as a indicator of physical performance as this outcome is directly related with fitness level and training status of the players (Krustrup *et al.*, 2003; Krustrup *et al.*, 2005). Recent findings, using computerised motion analysis, have shown that high-intensity running is reduced after the most intense periods of game-play and towards the end of the game (Mohr *et al.*, 2003, 2005), probably due to the effects of match-related fatigue.

### 2.2.3 Soccer-specific Skills

Apart from the spatial displacements on the field, soccer players perform a wide range of soccer-specific skills such as passing, dribbling, heading the ball and shooting. All these activities contribute to the total energy expenditure of soccer games. Successful soccer skilled actions are often important determinants of team success (Hughes and Franks, 2005) and contribute to the player's popularity. These soccer skills are also affected by fatigue during games. For example, Rampinini *et al.* (2009) demonstrated that physical and technical skills (involvements with the ball and short passing performance) declined during the second half of the game. Similar findings have been reported in adult semi-professional and academy players during a soccer simulation protocols (Russell *et al.*, 2011a; Stone and Oliver, 2009).

Most of soccer displacements and activities are predominantly performed at low- to moderate-intensity; therefore, the energy for these activities is mainly provided by aerobic metabolism. However, skilled actions (e.g. shooting, dribbling, tackling) and

high-intensity displacements mainly rely on anaerobic metabolism to provide the necessary amount of energy for muscle action. The energy requirements for soccer performance and the metabolic pathways that fuel physical activity during soccer games are addressed in Section 2.4.

## 2.3 Soccer-Specific Simulation Protocols

Soccer players perform 1000-1400 short activities changing every 4-6 seconds (Stolen *et al.*, 2005). This game variability makes practically impossible to replicate the activity patterns performed during actual soccer games. Several authors have designed different exercise protocols to replicate the demands of match performance (Clarke *et al.*, 2008; Drust *et al.*, 2000; Kingsley *et al.*, 2005; Nicholas *et al.*, 2000; Williams *et al.*, 2010). The main aim of these protocols is to dictate a similar and repetitive activity pattern over a period of time to produce comparable physiological responses. These soccer-specific protocols are often performed indoors, which permit the control of the environmental conditions.

Some of the soccer-specific protocols have replicated the activity patters of match-play using a non-motorised treadmill (Clarke *et al.*, 2008; Thatcher and Batterham, 2004). However, the unidirectional movements and the lack of soccer-specific skills within the protocol limited the ecological validity of this type of protocols. The Loughborough Intermittent Shuttle Test (LIST) was designed to replicate the physiological demands of soccer match-play (Nicholas *et al.*, 2000). The LIST consists of 75 min of shuttle running at variable intensities, followed by a continuous run, alternating 55% and 95% $\dot{V}O_{2\ max}$, until exhaustion. The LIST has been widely utilised to investigate the ergogenic potential of carbohydrate-electrolyte beverages (Morris *et al.*, 2003; Nicholas *et al.*, 1995; Nicholas *et al.*, 1999; Welsh *et al.*, 2002). However, the standard LIST did not included a half-time recovery period or soccer-specific skills, which reduced the ecological validity of the protocol.

More recent studies have used modified versions of the LIST, including a half-time period and additional multidirectional movements (lateral and backwards running) to

further replicate the demands of soccer games (Kingsley *et al.*, 2005; Russell *et al.*, 2011a). Additionally, studies by Ali *et al.* (2007) and McGregor *et al.* (1999) included soccer-specific skills before and after the protocol in a modified version of the LIST. However, the performance of soccer-specific skills before and after the protocol does not represent the true characteristics of match-play, where players are continuously involve with ball contacts (dribbling, passing, shooting, and heading). Ideally, a soccer-specific protocol should closely replicate the duration of a soccer game, including recovery periods and a fair representation of the movement patterns and soccer-specific skills performed during games. To this date, the soccer match simulation protocol (SMS) designed by Russell *et al.* (2011a) is the one that more closely represents game performance, incorporating a half-time recovery period and soccer-specific skills performed throughout the shuttle running protocol.

## 2.4 Energy Pathways in Soccer

Energy balance regulation during exercise is an extremely complex process including multiple interacting homeostatic and metabolic responses aimed at maintaining constant energy supply and stores (Niswender and Beech, 2008). The relative contribution of substrates (carbohydrates, lipids and proteins) oxidised during exercise mainly depends on the intensity and duration of the exercise bout (Romijn *et al.*, 1993), and the diet ingested before the exercise bout (Williams and Serratosa, 2006).

### 2.4.1 Aerobic Metabolism

Carbohydrates and lipids are the major fuels that enter the aerobic metabolic pathways to provide the energy source for the synthesis of the chemical fuel (Adenosine Triphosphate; ATP) that powers all biological work (mechanical, chemical and transport work). The normal duration of a soccer games is 90 min (120 min in certain competitions including extra-time periods); therefore, soccer is mainly dependent upon aerobic metabolism (Stolen *et al.*, 2005). The main substrates for aerobic metabolism during soccer activities are carbohydrates and lipids; however, proteins might provide

10% of the total energy required during an exercise bout similar to soccer match (Bangsbo, 1991; Wagenmakers *et al.*, 1991).

#### 2.4.1.1 Aerobic Metabolism from Carbohydrates

During moderate- to high-intensity exercise, the breakdown of glycogen or glucose (glycolysis) fuels the complex chain of reactions that lead to ATP formation. Glycolysis results in the production of pyruvate, which is irreversibly converted to acetyl Coenzyme A (acetyl CoA), a form of acetic acid. Acetyl CoA then enters the second pathway of carbohydrate (or lipids) metabolism, known as the citric acid cycle (or Krebs cycle). The oxidative production of ATP occurs in the inner lining of the mitochondrial membranes, where in the presence of oxygen, electrons are transfer from NADH and $FADH_2$ to oxygen, resulting in the release of chemical energy to synthesise ATP from ADP and an inorganic phosphate ion ($P_i$).

#### 2.4.1.2 Aerobic Metabolism from Lipids

Plasma free fatty acids are mainly utilised during prolonged and low- to moderate-exercise. Before the energy can be released from fat, the triacylglycerol molecule (fat molecule) needs to be broken down (lipolysis) into a molecule of glycerol and three fatty acids molecules. The circulating free fatty acids enter the cell and its carbon chain is cleaved into acetyl CoA molecules. From this point, the molecules of acetyl CoA obtained from the cleavage of fatty acids follow the same route as the molecules of acetyl CoA formed from the pyruvate molecules, entering the citric acid cycle to resynthesise ATP molecules. The glycerol molecules separated from the triacylglycerol breakage can enter the anaerobic glycolysis pathway to form 3-phosphoglyceraldehyde, which is then degraded into pyruvate. Glycerol can also enter the liver to provide carbon skeletons to synthesise glucose molecules (gluconeogenesis).

Nevertheless, fatty acids breakdown depends on a continuous supply of carbohydrates. When carbohydrate become depleted the rate of glycolysis is also reduced, and

therefore the amount of pyruvate formed is also reduced. Pyruvate is necessary for the formation of oxaloacetate, without which acetyl CoA cannot enter the citric acid cycle; therefore, reducing the activity of the citric acid cycle and the synthesis of ATP.

### 2.4.2 Anaerobic Metabolism

The energy for high-intensity activities during soccer games is mainly provided by the anaerobic pathways (anaerobic glycolysis, ATP-phosphocreatine system). Blood lactate concentration has been often used as an indicator of anaerobic glycolysis energy production during soccer. Early research showed large variations of blood lactate during soccer match-play, with average concentrations of 3-6 $mmol \cdot l^{-1}$ and peak values reaching 14 $mmol \cdot l^{-1}$ (Ekblom, 1986; Bangsbo, 1994; Reilly, 1997). Consequently, these findings indicate that the rate of anaerobic energy turnover is relatively high during games. It is important to consider the limitations when recording blood lactate during games, as differences have been observed between blood and muscle lactate concentrations, and blood lactate is dependent on the activity undertaken 5 min before the blood sample is taken (Krustrup *et al.*, 2006).

#### 2.4.2.1 The ATP-Phosphocreatine System

During sudden muscle activation, the change from rest or low-intensity exercise to high-intensity exercise is too rapid for an immediate external supply of energy to occur. Therefore, the stores of tissue ATP are utilised supplying energy for muscle activation during the first 2-4 s of exercise (McArdle *et al.*, 2006). The molecules of ATP are rapidly resynthesised from sarcoplasmic stores of phosphocreatine (PCr) if the exercise continues. Intramuscular PCr stores are nearly depleted after 15 to 30 s of high-intensity exercise and are only resynthesised during resting periods or low intensity exercise (Shephard, 1992). If PCr stores become nearly depleted, and no other energetic pathways supply ATP to maintain muscle contraction, fatigue will occur. In addition, muscle contraction is always associated with an increase in adenosine diphosphate (ADP) and inorganic phosphate ($P_i$). The concentration of these two metabolites (ADP

and $P_i$) increase during high-intensity exercise and higher levels of these metabolites have been suggested to inhibit the efficiency of muscle activity (Lionne *et al.*, 1995).

#### 2.4.2.2 Glycolysis

Glycolysis is activated at the onset of exercise and during high-intensity exercise to provide energy for muscle contraction as the energy demand exceeds the capacity for oxygen supply or its utilisation rate. Glycolysis produces ATP from the breakdown of carbohydrate molecules (glucose or glycogen). The end product of glycolysis is pyruvate that quickly captures the hydrogen ions transported by NADH to form lactate in the reaction helped by the enzyme lactate dehydrogenase. When the rate of glycolysis is high, lactic acid is form and accumulates in muscle cells and extracellular fluids, which leads to an increase in acidity. Some studies have established that a decrease in pH reduces isometric contraction force (Lamb and Stephenson, 1994) and muscle contraction velocity (Mainwood *et al.*, 1987). However, lactate should not be considered as a "metabolic wasted product". To the contrary, lactate acts as important metabolic intermediate for pyruvate formation, or can be synthesised to form glucose (glycogen) in the liver via the Cori cycle (McArdle *et al.*, 2006).

## 2.5 Fatigue in Soccer

Fatigue can be defined as an acute impairment in performance caused by an increase in the perceived effort necessary to produce a required force or movement and the eventual inability to produce this force (Lyons *et al.*, 2006). The onset of fatigue can be attributed to myriad factors and may occur at different times during soccer games.

### 2.5.1 Temporary Fatigue

Several studies have identified substantial transient decreases in high-intensity running after the most intense periods of the game (Mohr *et al.*, 2003; Bradley *et al.*, 2009). The technological advances in motion analysis have permitted a detailed quantification of

the high-intensity running patterns during soccer games. However, only a small number of studies have focused on identifying the causes behind the reductions in high-intensity exercise after intense periods in a soccer game. A study by Krustrup *et al.* (2006) revealed that muscle lactate was moderately elevated after intense periods of the game, with peak values reaching 15.9 ± 1.9 mmol·kg$^{-1}$ d.w. and 16.9 ± 2.3 mmol·kg$^{-1}$ d.w. in the first and second halves of game, respectively. However, muscle lactate concentrations did not correlate with the reduced sprint capacity during the game.

The decrease in muscle pH has been related to a reduction in exercise performance during soccer games (Ekblom, 1986). Nevertheless, muscle fatigue normally occurs when muscle pH equals 6.5, which represents the pH at which enzyme activity is inhibited (Jones *et al.*, 2004); muscle pH rarely reaches values below 6.8 during soccer games (Bangsbo *et al.*, 2007). Reduced muscle phosphocreatine (PCr) and ATP levels have also been related with a decreased ability to perform repeated sprints (Gaitanos *et al.*, 1993); however, this should be seen as an indicator of fatigue and not as the source of it. Additionally, potassium ($K^+$) accumulation in muscle interstitium has been linked with the onset of fatigue after high-intensity exercise (Fitts and Balog, 1996; Nielsen *et al.*, 2004). However, the actual cause of muscle fatigue may be related with the effects of $K^+$ accumulation in the depolarisation of the muscle sarcolemma rather to an accumulation of $K^+$ in the muscle interstitium (Mohr *et al.*, 2004b).

High-intensity exercise is impaired after periods of sustained bouts of high-intensity exercise. However, this type of temporary fatigue is likely to originate from a combination of disturbances in the muscle cell and changes in muscle metabolites that temporarily affect muscle contraction until a certain degree of homeostasis is recovered.

### 2.5.2 Fatigue during the Second Half

It is widely recognised that there is a decline in the total distance covered, the amount of high-intensity exercise and the amount of jogging performed during the second half of the match (Rampinini *et al.*, 2007; Bradley *et al.*, 2009; Di Salvo *et al.*, 2009; Vigne *et al.*, 2010; Carling and Dupont, 2011). Additionally, the distance covered by walking

and the recovery time between bouts of high-intensity exercise increase during the second half (Bangsbo and Krustrup, 2006; Bradley *et al.*, 2009; Vigne *et al.*, 2010). One of the possible causes of the decline in exercise performance towards the end of the game is the reduction of glycogen stores in muscle and liver. An early study by Saltin (1973) revealed that muscle glycogen stores were almost depleted at the end of the game (50 $mmol \cdot kg^{-1}$ d.w.) in soccer players that started the game with normal levels of muscle glycogen (~400 $mmol \cdot kg^{-1}$ d.w.). A later study by Krustrup *et al.* (2006) found that muscle glycogen was depleted or almost depleted in 47% of the leg muscle fibres after a soccer game. However not all studies have found such accentuated decreases in muscle glycogen after soccer games (Jacobs *et al.*, 1982).

Dehydration has been also associated with the decline in exercise performance towards the end of the game, particularly in hot and humid conditions (Maughan and Leiper, 1994; Reilly, 1997). Moderate losses in body water (2-3% BM) can negatively influence exercise (Edwards *et al.*, 2007) and skill performance (McGregor *et al.*, 1999) in soccer. The formation and evaporation of sweat plays an important role in protecting the body against hyperthermia (Maughan *et al.*, 2004). If body water losses are excessive, soccer performance can be jeopardised by a reduction of cardiovascular function and electrolyte imbalance. However, it is not clear if the loss of body water during exercise (sweat, respiration) is the direct cause of fatigue or is just part of a complex interaction of multiple physiological responses that cause the reductions in exercise performance (Lambert *et al.*, 2005).

The elevation of metabolic rate during exercise causes a rise in body temperature that can cause fatigue if the heat dissipation mechanisms are impaired. If exercise is performed in hot and humid conditions, the mechanism of heat dissipation are further stressed, which can cause the cessation of activity. It has been observed that exercise work-rate decreased during games played in hot environments (~30°C) (Ekblom, 1986; Mohr *et al.*, 2010). Additionally, Morris *et al.* (2005) showed a reduced exercise capacity and sprint performance during a soccer-specific protocol (LIST) performed in the heat (33 ° C) compared with moderate environmental conditions (17°C). Marked elevations in core temperature (2-3°C) have resulted in elevated skin blood flow and reductions in cardiac output, which can affect exercise performance (Gonzalez-Alonso

*et al.*, 1999). Additionally, exercise in the heat has been related with an increased rate of glycolysis and, therefore, an increased muscle glycogen utilisation (Fink *et al.*, 1975). Furthermore, high core temperatures can deteriorate cerebral function, which may provoke central fatigue (Nybo and Secher, 2004). The underlying factors behind the central fatigue hypothesis have been exposed elsewhere (Newsholme *et al.*, 1987). The origin of fatigue in soccer seems to be a complex mechanism influenced by a succession of peripheral and central events.

### 2.5.3 Fatigue after the Half-Time Period

Several studies have identified a decrease in exercise performance immediately after the half-time period (45-60 min) in comparison with the corresponding period (0-15 min) of the first half (Bangsbo *et al.*, 1991; Mohr *et al.*, 2003; Bradley *et al.*, 2009; Weston *et al.*, 2011). Some studies have attributed the lowered exercise performance seen during the first 15 min of the second half to the decline in muscle temperature due to the passive recovery strategy often utilised during the half-time period (Bangsbo, 1995). A study by Mohr *et al.* (2004a) showed that including a light-intensity warm-up (~70% peak heart rate) before the start of the second half of the game (lasting 7 min) reduced the decline in muscle and core temperature and maintained sprint performance. However, this strategy may negatively affect performance especially when games are played in hot environments as elevations in core temperature can be detrimental for exercise performance (Mohr *et al.*, 2010; Ozgünen *et al.*, 2010). Additionally, the tactical information given by the coach during the half-time recovery period is usually a valuable feedback of the team performance, and will required the player's full attention to process the information received.

A common practice among soccer players is to ingest fluids during the half-time recovery period to avoid dehydration, especially when games are played in hot conditions. Typically, carbohydrate drinks (sport drinks) are ingested during the half-time period to maintain a stable glucose concentration and promote exogenous carbohydrate utilisation (Karelis *et al.*, 2010). However, several studies have observed that carbohydrate ingestion before exercise can result in an acute hyperinsulinaemic

response followed by an acute drop in blood glucose at the onset of exercise (Sherman *et al.*, 1991; Kuipers *et al.*, 1999). In agreement with the above mentioned studies, pilot observations carried out prior to the present study revealed that the ingestion of carbohydrate drinks (6%) during the half-time period significantly reduced blood glucose concentration after the half-time recovery period (Appendix H). Low levels of blood glucose during the first 15 min of the second half might be related to reductions in exercise and soccer skill performance. The decline in blood glucose concentration after carbohydrate drinks ingestion might reflect high insulin concentrations, increased muscle glucose uptake and reduced liver glucose output (Marmy-Conus *et al.*, 1996). As reductions in blood glucose levels during the first instances of the second half might be accompanied by a decrease in soccer performance, future studies should be focused to investigate possible nutritional strategies to attenuate the blood glucose decrease during the initial stages of the second half of the match.

## 2.6 Carbohydrate Ingestion in Soccer

Fatigue in soccer has been widely associated with the depletion of muscle glycogen, hypoglycaemia, hypohydration and hyperthermia (Mohr *et al.*, 2005). The ingestion of carbohydrate has advocated the maintenance of homeostasis during exercise, by preserving an optimal level of carbohydrate for muscle and brain metabolism. Additionally, the ingestion of carbohydrate drinks might help to maintain an adequate water balance during exercise. Therefore, the ingestion of carbohydrate has been widely recommended to attenuate the effects of fatigue during soccer activities (Casa *et al.*, 2000; Sawka *et al.*, 2007). The effects of carbohydrate ingestion during match-play and soccer-specific protocols have been widely investigated (Table 2.1).

The majority of studies included in Table 2.1 have associated carbohydrate ingestion with performance improvements during high-intensity intermittent exercise (Ali *et al.*, 2007; Nicholas *et al.*, 1995; Ostojic and Mazic, 2002; Phillips *et al.*, 2010). However, some studies failed to identify benefits associated with carbohydrate supplementation (Morris *et al.*, 2003; Zeedeberg *et al.*, 1996), probably due to methodology variations (e.g. definition of successful performance, blinding procedures).

**Table 2.1** Previous studies that have investigated the influence of carbohydrate supplementation on soccer performance

| Study | Solution | Rate/Time of Ingestion | Situation | Effect of CHO |
|---|---|---|---|---|
| Leatt and Jacobs, (1989) | 7% glucose polymer or flavoured water | 0.5 l 10 min Pre-game<br>0.5 l at half-time | Match-play | Reduced muscle glycogen depletion |
| Nicholas *et al.*, (1995) | 6.9% carbohydrate-electrolyte or flavoured placebo | 5 ml·kg$^{-1}$ Pre-exercise<br>2 ml·kg$^{-1}$ Every 15 min during exercise | Soccer protocol (LIST) | No effect on 15-m sprints<br>Prolonged time to exhaustion |
| Zeedeberg *et al.*, (1996) | 6.9% glucose polymer or flavoured water | 5 ml·kg$^{-1}$ 15 min Pre-game<br>5 ml·kg$^{-1}$ at half-time | Match-play | No benefits in motor soccer performance during game |
| Ostojic and Mazic, (2002) | 7% carbohydrate-electrolyte or placebo | 5 ml·kg$^{-1}$ Pre-exercise<br>2 ml·kg$^{-1}$ Every 15 min during exercise | Match-play | Improved soccer-specific performance |
| Morris *et al.*, (2003) | 6.5% carbohydrate-electrolyte, flavoured water and a flavoured Na solution | 6.5 ml·kg$^{-1}$ 15 min Pre-exercise<br>4.5 ml·kg$^{-1}$every 19 min during exercise | Soccer protocol (modified LIST) | No improvement in time to exhaustion<br>No effect on 15-m sprints |
| Ali *et al.*, (2007) | 6.4% carbohydrate-electrolyte or flavoured placebo | 5 ml·kg$^{-1}$ Pre-exercise<br>2 ml·kg$^{-1}$ Every 15 min during exercise | Soccer protocol (LIST) | Faster mean sprint performance<br>Higher final blood glucose |
| Backhouse *et al.*, (2007) | 6.4% carbohydrate-electrolyte or flavoured placebo | 8 ml·kg$^{-1}$ Pre-exercise<br>3 ml·kg$^{-1}$ Every 15 min during exercise | Soccer protocol (LIST) | Higher plasma glucose and reduced rate of perceived exertion |
| Patterson and Gray, (2007) | CHO gel ingestion vs. flavoured placebo beverage | 0.89 ml·kg$^{-1}$ Pre-exercise<br>0.35 ml·kg$^{-1}$ Every 15 min during exercise | Soccer protocol (LIST) | Prolonged time to exhaustion. Similar physiological response between trials |
| Foskett *et al.*, (2008) | 6.4% carbohydrate-electrolyte or placebo after diet rich in carbohydrate | 8 ml·kg$^{-1}$ Pre-exercise<br>3 ml·kg$^{-1}$ Every 15 min during exercise | Soccer protocol (LIST) | Prolonged time to exhaustion<br>Higher glucose levels at fatigue |
| Currel *et al.*, (2009) | 7.5% maltodextrin solution and placebo beverage | 6 ml·kg$^{-1}$ 30 min prior exercise<br>4 ml·kg$^{-1}$ at half-time, 1 ml·kg$^{-1}$ every 12 min during exercise | Soccer protocol | Improved scores in dribbling, agility and shooting |
| Ali and Williams, (2009) | 6.4% carbohydrate-electrolyte or flavoured placebo | 8 ml·kg$^{-1}$ Pre-exercise<br>3 ml·kg$^{-1}$ Every 15 min during exercise | Soccer protocol (LIST) | No difference in sprint performance. Similar physiological response between trials |
| Phillips *et al.*, (2010) | 6% carbohydrate-electrolyte or a flavoured placebo | 5 ml·kg$^{-1}$ 5 min Pre-exercise<br>2 ml·kg$^{-1}$ Every 15 min during exercise | Soccer protocol (LIST) | No effect on 15-m sprints<br>Increased time to exhaustion |
| Phillips *et al.*, (2011) | CHO gel ingestion vs. placebo gel | 0.82 ml·kg$^{-1}$ Pre-exercise<br>0.33 ml·kg$^{-1}$ Every 15 min during exercise | Soccer protocol (LIST) | Prolonged time to exhaustion<br>No effect on 15-m sprints |

The comparison of the findings observed between studies is complex due to a large variation in the methods employed. For example, most of the studies used the co-ingestion of carbohydrate-electrolyte beverages, while others used carbohydrate gels (Paterson and Gray, 2007; Phillips *et al.*, 2011) to investigate the ergogenic effects of carbohydrate supplementation during high-intensity intermittent exercise. In addition, differences exist in the time of ingestion and the amount of fluid ingested in the study. The majority of studies provided an initial bolus of fluid (5-8 $ml \cdot kg^{-1}$) prior to exercise, followed by the successive ingestion of 2-4.5 $ml \cdot kg^{-1}$ of fluid every 15 min during exercise. However, some studies have provided a similar amount of fluids (5 $ml \cdot kg^{-1}$ or 500 ml) before the start of the exercise bout and during the half-time recovery period (Leatt and Jacobs, 1989; Zeedeberg *et al.*, 1996). Additionally, the composition of the beverage such as, the carbohydrate concentration, type of carbohydrate, electrolyte concentration, solution osmolality and use of additional compounds (caffeine) varied significantly across studies and will be discussed in Section 2.7. These variations within the literature make it difficult to elucidate the optimal strategy of carbohydrate supplementation during high-intensity intermittent sports and future research of these areas is required to identify the optimal formula.

### 2.6.1 Forms of Ingested Carbohydrate

Carbohydrates are generally ingested as fluids because this helps to maintain optimal hydration status and blood glucose concentration, and spares muscle glycogen during exercise (Millard-Stafford *et al.*, 1992; Nicholas *et al.*, 1995; Tzintzas *et al.*, 1993). However, the effect of ingesting different forms of carbohydrate (semi-solids or solids) on metabolism and performance is not fully understood.

The ingestion of a similar dose of carbohydrate in a liquid or a solid form has resulted in similar metabolic responses and similar performance improvements during prolonged cycling (Campbell *et al.*, 2008; Lugo *et al.*, 1993; Pfeiffer *et al.*, 2010). To date, only two studies have investigated the effects of carbohydrate gels ingestion during soccer-specific protocols. A study by Patterson and Gray (2007) showed that the ingestion of an isotonic carbohydrate gel (43 $g \cdot h^{-1}$ of CHO) along with water, maintained blood

glucose levels and improved time to exhaustion during an intermittent high-intensity exercise protocol. Similarly, Phillips *et al.* (2011) revealed that the ingestion of a carbohydrate gel (0.78 $g \cdot kg^{-1}$ of BM) along with water increased time to exhaustion but did not influence sprint performance during an intermittent high-intensity exercise protocol. This study, however, did not investigate the metabolic responses during exercise so the reasons behind the exercise improvement were not clearly defined. Therefore, further studies should investigate the effects of carbohydrate gels ingestion on metabolism and exercise performance during soccer activities to increase the evidence of the beneficial effects of carbohydrate gels ingestion during exercise.

### 2.6.2 Time of Carbohydrate Ingestion

The foods and fluids ingested in the hours before training and competition and during exercise can influence performance by reducing the effects of fatigue (Williams and Serratosa, 2006). Adequate food and fluid ingestion before and during exercise helps to maintain blood glucose concentration, providing energy for muscle and brain metabolism (Meeussen *et al.*, 2006). It has been suggested that players should ingest a high carbohydrate meal 3 hours before exercise, and sports drinks prior and during exercise, to reduce the effects of fatigue (FIFA, 2006; Williams and Serratosa, 2006). However, several studies investigating the ergogenic effects of carbohydrate supplementation in soccer performance have utilised a combination of glycogen depleting routines with low carbohydrate meals and overnight fasting prior to the main trials, which contradicts the current recommendations and reduce the ecological validity (Ali *et al.*, 2007, Ali and Williams, 2009; Nicholas *et al.*, 1999; Nicholas *et al.*, 1995; Phillips *et al.*, 2010). In the current study, participants' intake of carbohydrates was not manipulated previous the exercise protocol. Additionally, a high carbohydrate breakfast was ingested 2 hours before the exercise protocol, along with 500 ml of the treatment, routine that followed the nutritional current practices and increased the ecological validity of the study (Sawka et al., 2007; Williams and Serratosa, 2006).

The opportunities to ingest fluids and carbohydrates during soccer games are limited and depend on the amount substitutions, free-kicks and other stoppages that occur

during the game. For this reason, it is recommended that soccer players start games with high levels of muscle glycogen and in a euhydrated status. The only secure opportunities in which players can ingest fluids and carbohydrates during competition are before kick-off and at half-time. Therefore, optimal strategies for fluid and energy replacement should be placed around these times. An early study by Leatt and Jacobs (1989) revealed that ingesting a 500 ml of a 7% glucose polymer drink 10 min before a soccer game and at half time reduced muscle glycogen utilisation, mainly by maintaining blood glucose levels. More recent studies have used a different fluid ingestion strategy, in which fluids (carbohydrate beverages) are ingested before kick-off and consecutively during exercise (Table 2.1).

Although the beneficial effects of fluid and carbohydrate ingestion previous and during exercise have been widely reported, some studies have also shown that carbohydrate ingestion within the hour before exercise may result in a drop of blood glucose at the onset of exercise (Kuipers *et al.*, 1999; Sherman *et al.*, 1991). This transient drop in blood glucose concentrations (hypoglycaemia) might not influence the overall exercise performance (Chryssanthopoulos *et al.*, 2002); however, hypoglycaemia (glucose concentration $< 3.5$ $mmol \cdot l^{-1}$) has been associated with the deterioration of both cognitive (Benton, 2002; Nybo, 2003) and physical performance (Ali *et al.*, 2007; Coyle *et al.*, 1986; Welsh *et al.*, 2002). Therefore, low glucose concentrations during the initial stages of the second half might decrease exercise and skill performance. Therefore, soccer players should follow a carbohydrate rich diet before training and competition (2-3 hours prior exercise), combined with the ingestion of fluid and carbohydrates during exercise to maintain a stable blood glucose concentration. The ingestion of carbohydrate beverages during the half-time period might attenuate the blood glucose drop observed after short periods of inactivity within a bout of exercise.

### 2.6.3 Participants

To date, most of studies have used elite or first-class soccer players to investigate the effects of dehydration and carbohydrate ingestion during soccer-specific activities (Edwards *et* al., 2007; Maughan *et al.*, 2005; Silva *et al.*, 2011). However, recreational

or young individuals could be considered, in some cases, as potential elite or professional player. Therefore, it would be interesting to identify the physiological differences between young or recreational individuals and their elite colleagues. Additionally, the majority of registered soccer players practice the sport in a recreational way; therefore more attention should be paid to the nutritional and hydration needs of different populations such as recreational and female players during soccer activities.

## 2.7 Carbohydrate Ingestion and Hydration

Muscle glycogen depletion and low blood glucose concentrations have been widely associated with the onset of fatigue during prolonged exercise (>60 min). Additionally, excessive hypohydration (>2-3% BM) can impair soccer-specific performance and increase the probability of suffering heat related illnesses (Edwards *et al.*, 2007; Maughan *et al.*, 2004; Mcgregor *et al.*, 1999). The aim of ingesting fluids during exercise is to replace water and electrolyte losses so exercise and skills performance can be sustained during the total duration of the exercise bout (training and competition). Additionally, the ingestion of fluids containing carbohydrates might increase or maintain exercise and skill performance if fuel delivery is maximised without compromising fluid delivery. In order to provide an optimal fluid and fuel delivery during exercise, the ingested carbohydrate solution should be quickly emptied from the stomach and absorbed in the small intestine so the nutrients can be delivered into the bloodstream. However, there are several factors that can affect the rates in which fluid and carbohydrate are absorbed in the digestive system such as the composition, volume, concentration and osmolality of the solution ingested and the exercise intensity (Shi and Gisolfi, 1998; Gisolfi, 2000).

### 2.7.1 Fluid Volume

Exercise-induced dehydration can result in a raise in core temperature (Cheuvront *et al.*, 2004), increased cardiovascular stress (Montain and Coyle, 1992), increased muscle glycogen utilisation (Hargreaves *et al.*, 1996), increased perception of effort (Montain

and Coyle, 1992), and cause reductions in blood flow towards the skin (Gonzalez-Alonso *et al.*, 1998). It has been observed that water losses above 2% of body mass can negatively affect soccer-specific exercise performance (Edwards *et al.*, 2007; McGregor *et* al., 1999). Therefore, professional soccer players should ingest sufficient fluids during training and competition to limit sweat losses to about 2% of body mass (Shirreffs *et al.*, 2006). Body mass losses are affected by environmental conditions, with players losing 1-2 kg during games played in temperate conditions (Maughan *et al.*, 2007b, Shirreffs, 2010), with more important losses (3-4 kg) during games played in hot conditions (Kurdak *et al.*, 2010; Mustafa and Mahmoud, 1979). Additionally, it has been observed that there is a great variability in water losses among players exercising in similar conditions (Maughan *et al.*, 2004; 2005; Shirreffs *et al.*, 2005). For these reasons, fluid ingestion during training and competition should be adapted to the environmental conditions and to the individual characteristics of the players (Shirreffs *et al.*, 2006).

The amount of fluid ingested can influence the rate in which fluids and carbohydrates are emptied from the stomach and absorbed in the small intestine. Several studies have shown that fluid and nutrient absorption is enhanced when the volume of fluid ingested is increased, at rest or during prolonged steady-state exercise (Duchman *et al.*, 1997; Rehrer *et al.*, 1990). However, the ingestion of fluids in excess before and during exercise can produce gastro-intestinal discomfort, affecting exercise performance (Reilly and Ekblom, 2005). A study by Clarke *et al.* (2008) demonstrated that manipulating the timing and volume of ingestion of a carbohydrate drink elicited the same metabolic responses if the total volume of fluid ingestion was maintained. Furthermore, the study showed that ingesting a small volume of fluid at regular intervals during exercise reduced the sensation of gut fullness, compared with the ingestion of a large volume of fluid at a specific time (half-time). Therefore, the fluid intake recommendations during exercise should take into consideration the environmental conditions and the characteristics of the individual (sweat rates, heat acclimatisation, genetics, and fitness).

### 2.7.2 Exercise Type.

The type and intensity of a bout of exercise might influence the rate of fluid and fuel absorption during exercise. It has been suggested that high-intensity intermittent exercise delays the gastric emptying of fluids ingested compared with a similar exercise bout of continuous exercise (Leiper *et al.*, 2001a) or a low-intensity walking protocol (Leiper *et al.*, 2005). Additionally, Leiper *et al.* (2001b) showed that gastric emptying was negatively affected during a 5-a-side indoor soccer match. However, the exercise characteristics of an 11-a-side soccer match permits soccer players to exercise at low intensities for large periods of time so gastric emptying of fluids might not be significantly affected.

### 2.7.3 Sport Drinks Composition

#### 2.7.3.1 Type of Carbohydrate

Carbohydrates are most typically incorporated in sport drinks as monosaccharides (glucose and fructose), disaccharides (sucrose), and synthetic glucose polymers (maltodextrin). Glucose is quickly absorbed within the first 20% of the small intestine (Widmaier *et al.*, 2011) from the intestinal lumen by secondary active transport coupled to sodium (sodium dependent glucose transporters-SGLT1) and subsequently diffused into the blood circulation for oxidation or storage. It has been suggested that glucose oxidation rate (~1.1 $g \cdot min^{-1}$) is mainly limited by the rate of absorption in the intestinal lumen as the transport proteins (SGLT1), which can become saturated when glucose is ingested at rates higher than 1.2 $g \cdot min^{-1}$ (Ferraris, 2001; Jentjens *et al.*, 2004).

Fructose, in contrast to glucose, is absorbed from the intestinal lumen and facilitated via the transport protein GLUT5 (Jentjens *et al.*, 2004). Several studies have reported lower oxidation rates of fructose compared with glucose (Adopo *et al.*, 1994; Jandrain *et al.*, 1993; Massicotte *et al.*, 1994)), probably due to a lower rate of absorption and that

fructose needs to be converted to glucose in the liver before it is metabolically available (Jentjens *et al.*, 2004; Jeukendrup and Jentjens, 2000).

Ingested disaccharides (sucrose) are broken down into monosaccharide molecules (glucose, fructose) by enzymes located in the luminal membrane of the small intestine. An early study by Wagenmakers *et al.* (1993) showed that sucrose is oxidised at similar rates as glucose (0.87 $g \cdot min^{-1}$) so the metabolic efficacy of glucose and sucrose ingestion may be similar. However, Jentjens *et al.* (2005b) showed that the carbohydrate oxidation rates were higher with the ingestion of sucrose compared with the ingestion of a similar amount (1.2 $g \cdot min^{-1}$) of glucose during prolonged continuous cycling.

Maltodextrin is a glucose polymer that is hydrolysed in the upper part of the small intestine into glucose molecules before following the same pathway as glucose. Maltodextrin is commonly included in commercial drinks due to its neutral taste (improving palatability) and relative low osmolality compared with glucose; consequently, manufacturers can increase the carbohydrate content of the drinks. Several studies have observed similar oxidation rates when similar amounts of maltodextrin and glucose were ingested during continuous cycling exercise (Massicotte *et al.*, 1989; Rehrer *et al.*, 1992). Wallis *et al.* (2005) demonstrated that the ingestion of a large dose of combined maltodextrin and fructose produced higher carbohydrate oxidation rates than the equivalent ingestion of maltodextrin.

Although carbohydrate oxidation might be limited by the rate of gastric emptying and the rate of glucose storage by the liver and muscle (Hulston *et al.*, 2009), studies have suggested that carbohydrate oxidation is mainly limited by intestinal carbohydrate absorption (Jentjens and Jeukendrup, 2005a; Jentjens *et al.*, 2006). The combined ingestion of carbohydrate molecules such as glucose and fructose (Jentjens *et al.*, 2004), glucose and sucrose (Jentjens *et al.*, 2005b), maltodextrin and fructose (Wallis *et al.*, 2005) can increase the rate of carbohydrate oxidation up to 1.2-1.5 $g \cdot min^{-1}$. This probably occurs because glucose and fructose do not compete for the same protein transporters therefore more carbohydrate can be transported into the blood stream for oxidation. A study by Shi *et al.* (1995) suggested that the inclusion of two or three

different molecules of carbohydrate in a sports drink could increase water and carbohydrate absorption despite the increased osmolality.

Most of the studies investigating the effects of ingesting multiple transportable carbohydrates on carbohydrate metabolism during exercise have used prolonged continuous exercise protocols. Therefore, it would be of value to investigate the effects of ingesting multiple transportable carbohydrates during high-intensity intermittent activities, such as soccer.

### 2.7.3.2 Electrolytes

The high metabolic rates sustained by soccer players during training and games-play can cause significant sweat losses (Maughan *et al.*, 2004; Maughan *et al.*, 2005). However, large differences in sweat loss have been observed among individuals exercising at similar exercise intensities and environmental conditions (Shirreffs *et al.*, 2005). Different electrolytes are lost through sweat (potassium, calcium, magnesium and chloride) but maintaining the sodium ($Na^+$) balance is fundamental because $Na^+$ is the major cation present in the extra-cellular space (Maughan *et al.*, 2005). A decrease in sodium concentration (hyponatraemia) below 135 $mmol \cdot l^{-1}$ can result in health problems. The most common symptoms associated with low levels of sodium are headaches, muscle crumps, nausea and seizures; however, if very low levels are reached ($<125$ $mmol \cdot l^{-1}$) a lethal rapid influx of water into the brain can occur (McArdle *et al.*, 2006). Exercise-induced hyponatraemia can be caused by a combination of factors such as, an excessive ingestion of water or hypotonic fluids, an excessive loss of plasma sodium through sweat, and high concentrations of antidiuretic hormone (ADH).

Although exercise-induced hyponatraemia has not been frequently reported during soccer games (Shirreffs *et al.*, 2006), the addition of sodium to sport drinks is intended to replace the sodium loss through sweat (Montain *et al.*, 2006), and to stimulate carbohydrate and water uptake in the small intestine and maintain the drive to ingest fluids (Maughan *et al.*, 2007).

### 2.7.3.3 Caffeine

Caffeine (1, 3, 7-trimethylxantine) is an alkaloid molecule that is rapidly emptied from the stomach and absorbed in the intestinal tract (Jacobson and Kulling, 1989). Caffeine is also quickly metabolised by the liver so its concentration in blood reaches peak values approximately one hour after ingestion (Harland, 2000). The ergogenic properties of caffeine have been associated with several mechanisms. For example, caffeine and its metabolites (paraxanthine, theophylline and theobromine) cross the blood-brain barrier without difficulty, producing an analgesic effect that may reduce perception of effort and muscle pain during exercise (Fredholm *et al.*, 1999; Motl *et al.*, 2006). Caffeine ingestion has been also associated with motor unit recruitment enhancements due to the blockage of adenosine, a neuro-modulator that calms brain and spinal cord neural activity (Spriet and Gibala, 2004; Sokmen *et al.*, 2008). Some authors have suggested that caffeine decreases the use of glycogen by increasing fatty acids availability during intense, endurance exercise (Ivy *et al.*, 1979; Spriet *et al.*, 1992). The ingestion of caffeine along with carbohydrates might increase the rate of exogenous carbohydrate oxidation during exercise; possibly as a result of an enhanced intestinal absorption (Yeo *et al.*, 2005; Van Nieuwenhoven *et al.*, 2000).

Caffeine should be ingested with caution as high doses of caffeine (>6 $mg \cdot kg^{-1}$) intake may have adverse health effects, in particular, affecting cardiovascular function (Tarnopolsky, 1994). Additionally, caffeine ingestion might act as a diuretic; therefore, individuals have been discouraged from consuming caffeine during activities where hydration can be compromised (Neuhauser-Berthold *et al.*, 1997; Passmore *et al.*, 1987). However, several studies have indicated that the ingestion of moderate doses of caffeine (3-6 $mg \cdot kg^{-1}$ BM) did not compromise hydration status during prolonged exercise (Millard-Stafford *et al.*, 2007; Armstrong *et al.*, 2007; Del Coso *et al.*, 2009).

The use of caffeine in sport drinks is relatively new because of the alleged diuretic effects and its inclusion in the International Olympic Committee (IOC) list of banned substances until 2004. The acute ingestion of caffeine (3-13 $mg \cdot kg^{-1}$) has produced improvements in endurance exercise (Costill *et al.*, 1978; Graham and Spriet, 1991;

Pasman *et al.*, 1995), sprint performance (Glaister *et al.*, 2008) and soccer skills performance. The ingestion of caffeinated carbohydrate beverages have resulted in endurance performance improvements (Cox *et al.*, 2002; Kovacs *et al.*, 1998). Additionally, a study by Gant *et al.* (2010) showed that the co-ingestion of carbohydrate (6% carbohydrate beverage) and caffeine (160 $mg \cdot l^{-1}$) improved sprint and countermovement jump performance of a group of professional soccer players during a 90-min soccer-specific protocol. However, several studies have failed to report performance benefits associated with the ingestion of caffeinated carbohydrate beverages (Desbrow *et al.*, 2009; Hunter *et al.*, 2002; Jacobson *et al.*, 2001).

The disparity in results between studies probably reflects variations in the doses of caffeine and carbohydrates administered, and the intensity, duration and the type of exercise performed in the respective studies. Several studies have associated caffeine ingestion with an increase in exogenous carbohydrate absorption and oxidation. However, most studies have used a carbohydrate dose (6-7% carbohydrate solutions) that did not exceed the glucose oxidation rate limit (1.1 $g \cdot min^{-1}$) (Jentjens *et al.*, 2004). Therefore, it would be interesting to investigate the effects of caffeine ingestion with higher doses of carbohydrates (8-10% carbohydrate) during soccer-specific activities.

Moreover, most of the studies reviewed have utilised well-trained male athletes, overlooking different population groups such as, female athletes, recreational players and mature athletes. This is surprising as recreational players are probably the major consumers of carbohydrate drinks and ergogenic supplements, so the health and performance effects of these nutritional supplements should be well documented for this population.

### 2.7.4 Carbohydrate Concentration

The carbohydrate concentration of the most popular commercially available sport drinks range between 6% and 8% (Coombes and Hamilton, 2000). This beverage formulation follows the American College of Sports Medicine (ACSM) recommendations (500-1000 $ml \cdot h^{-1}$ providing 30-80 $g \cdot h^{-1}$ of carbohydrate) for carbohydrate and fluid intake

during prolonged exercise (Sawka *et al.*, 2007). However, this recommendation does not differentiate between prolonged intermittent and continuous exercise.

Some studies have investigated the effects of ingesting beverages with large doses of carbohydrate (8-18%) during prolonged continuous exercise and these have resulted in exercise improvements in some of the studies (Mitchell *et al.*, 1989) but not all (Flynn *et al.*, 1987; Galloway *et al.*, 2001). The ingestion of a 17% glucose solution (220 g of carbohydrate) during 80 min cycle at 70% $\dot{V}O_{2\ max}$ resulted in higher carbohydrate oxidation when compared with the ingestion of a 4.5% glucose solution (58 g). Similarly, Wagenmarkers *et al.* (1993) administered 4% (0.6 $g \cdot min^{-1}$), 8% (1.2 $g \cdot min^{-1}$), 12% (1.8 $g \cdot min^{-1}$) and 16% (2.4 $g \cdot min^{-1}$) maltodextrin solutions during a 120 min cycle exercise at 65% $\dot{V}O_{2\ max}$ , resulting in an increase of carbohydrate oxidation with increased intake.

The ingestion of high doses of carbohydrate during prolonged exercise can maintain blood glucose levels, attenuate the rate of endogenous carbohydrate production (Jeukendrup *et al.*, 1999), increase carbohydrate oxidation, and in some cases, improve exercise performance (Pfeiffer *et al.*, 2011). However, some studies have found that ingesting high doses of carbohydrate may reduce gastric emptying rate (Costill and Saltin, 1974; Rehrer *et al.*, 1992; Vist and Maughan, 1994) and intestinal water absorption (Gisolfi *et al.*, 1992), which can increase the chances of suffering gastrointestinal disturbances (Brouns and Beckers, 1993; Rehrer *et al.*, 1993; Pfeiffer *et al.*, 2011) and water imbalances during exercise.

Despite the possible benefits and disadvantages of ingesting a high concentration carbohydrate drink, only a limited number of studies have investigated its effects during intermittent exercise (Murray *et al*, 1989; Welsh *et al.*, 2002). Also, the methodology utilised in some of these studies offer little application for intermittent sports. To this date, only one study (Phillips *et al.*, 2011) has investigated the effects of ingesting carbohydrate beverages containing different carbohydrate concentrations (2, 6 and 10%) on intermittent endurance capacity and sprint performance during a soccer-specific protocol. This study showed that the ingestion of a 6% carbohydrate solution improved

intermittent endurance capacity compared with the 10% solution. Additionally, there was a tendency of improvement of endurance capacity with the ingestion of the 2% solution compared with the 10% ($P = 0.09$). However, the reasons behind the improved performance in the 6% solution were not clearly explained as the majority of the variables investigated (body mass losses, sweat rates, RPE, abdominal discomfort rates) were similar across all trials. Therefore, the investigation of metabolic parameters (blood glucose and lactate responses) and further hydration assessments might offer additional information to explain the reasons behind performance improvements during high-intensity intermittent protocols.

### 2.7.5 Solution Osmolality

The osmolality in a solution determines the osmotic gradient that will influence the movement of water across the intestinal wall and, therefore, determine the direction and rate of fluid movement in the small intestine (Evans *et al.*, 2009). An adequate fluid absorption from the intestinal lumen and a rapid carbohydrate delivery to the systemic circulation are important features of a good quality sport drink, among palatability and carbohydrate concentration. Several studies have suggested that fluid absorption in the intestinal lumen is inversely related to the osmolality of the carbohydrate solution (Hunt *et al.*, 1992; Gisolfi *et al.*, 1992; Shi *et al.*, 1995). However, Gisolfi *et al.* (1998) observed that varying the osmolality of a 6% carbohydrate drinks, with osmolalities ranging between 197-414 mosmol·kg$^{-1}$ did not influence total intestinal water absorption or fluid homeostasis during exercise. Similarly, Shi *et al.* (1994) showed that the perfusion of a 6% carbohydrate solution with osmolalities ranging from 186-403 mosmol·kg$^{-1}$ did not produced significant differences in fluid homeostasis during exercise.

The osmolality of the sport drink is mainly affected by concentration of carbohydrate and electrolytes, and the type of carbohydrate contained in the drink (Gisolfi *et al.*, 1992). Many of the commercially available sport drinks are hypertonic (300-500 mosmol·kg$^{-1}$) with concentrations of carbohydrate ranging from 5 to 8% (Coombes and Hamilton, 2000). In order to enhance fluid absorption, some of the brands have utilised

glucose polymers (maltodextrin) because larger doses of carbohydrates can be added to the drink without significantly increasing the solution osmolality (Shi and Gisolfi, 1998). Sport drinks with a high concentration of carbohydrates provide higher doses of exogenous carbohydrates for muscle utilisation; however, this may result in gastrointestinal discomfort (Brouns *et al.*, 1995). Additionally, Lambert *et al.* (2008) observed that the ingestion of multiple-transportable carbohydrates increased the rate of fluid absorption compared with the same amount of single-source carbohydrate ingestion. This was probably caused by the use of multiple transportable carbohydrates as this may counteract the effects of hyperosmolality on intestinal fluid absorption (Shi and Passe, 2010).

To date, studies have not investigated the hydration effects of a hypertonic, high carbohydrate concentration drink during intermittent high-intensity exercise; therefore future research should examine the effect of these types of carbohydrate supplementation during soccer-specific activities.

### 2.8 Hydration Measurements

The high metabolic rates sustained by players during training and competition increases the amount of body water losses, especially in hot conditions (Shirreffs *et al*, 2006). Reductions in body water equivalent or higher than 2% of body mass have been related with decreases of physical capacity (Edwards et al., 2007) and soccer skill performance (McGregor *et al.*, 1999). Therefore, soccer players should start the exercise bout (training or match) in a euhydrated status with normal levels of electrolytes in plasma and ingest an optimal amount of fluids and electrolytes to avoid water losses higher than 2% of body mass (Sawka *et al.*, 2007; Shirreffs *et al*, 2006). In order to confirm the players' hydration status previous to, during and after exercise and to ensure the adequate ingestion of fluids during exercise, several field- and laboratory based techniques can be utilised to assess hydration status. The use of the different methods will vary depending on the characteristics of the individual, and the facilities and equipment available for the measurement.

### 2.8.1 Changes in Body Mass

The measurement of acute changes in body mass over a short period of time (1-4 hours) is one of the most utilised field-based techniques to assess hydration status (Kavouras, 2002). Players should be weighed nude or with minimal clothes before and after training, exercise protocol or games (Shirreffs, 2010). It is assumed that the weight loss during exercise equals the amount of water loss, where a mass loss of 1 kg of mass approximates to 1 l of water loss. However, several factors should be considered in order to produce accurate measurements of body mass change. During exercise, water is lost not only in the form of sweat, but some water is also evaporated from the skin, and lost in respiration and as urine (Maughan and Shirreffs, 2010). Additionally, corrections should be performed to account for the amount of fluids and solid foods ingested, and the water generated as a by-product of substrate oxidation. Some of these factors can generally be ignored (respiration losses and substrate oxidation water gain) when sweat rates are high (Maughan *et al.*, 2007a); consequently, after correction for fluid and solid ingestion, the acute change in mass during exercise approximates to sweat loss. Additionally, a single measure of body mass cannot give an adequate indication of hydration status because morning body mass fluctuates by ~1% in well hydrated individuals who are in energy balance (Sawka *et al.*, 2007).

### 2.8.2 Plasma Osmolality

Plasma or serum osmolality is the most widely used haematological index of hydration status, as extra-cellular fluid osmolality activates important water homeostatic mechanisms (Armstrong, 2005). Plasma osmolality will normally increase as a result of water loss by sweat secretion, urine production, vomit or diarrhoea (Shirreffs, 2003). It has been suggested that an individual is euhydrated when plasma osmolality range between 280 and 300 mosmol·kg$^{-1}$ (Hamilton and Bickle, 2006; Weinberg and Minaker, 1995). A study by Popowski *et al.* (2001) indicated that plasma osmolality measures were sensitive to modest changes in hydration status during exercise. However, it has been suggested that plasma osmolality measures are subject to variations in posture, exercise intensity, and food and fluid intake (Maughan and Shirreffs, 2010).

### 2.8.3 Urine Osmolality

The collection of urine sample and subsequent osmolality analysis has been frequently used in sport settings to measure hydration status (Armstrong *et al.*, 1998; Shirreffs and Maughan, 1998). The American College of Sports Medicine suggests that a urine osmolality previous to exercise ≤700 mosmol·kg$^{-1}$ is indicative of euhydration (Sawka *et al.*, 2007). A urine osmolality of more than 900 mosmol·kg$^{-1}$ can be representative of a water deficit of ~2% of body mass (Shirreffs and Maughan, 1998). If urine osmolality is measured before exercise, it may offer a valuable estimate of hydration status (Sawka *et al.*, 2007). However, urine osmolality is not a sensitive measure of hydration status after exercise (Kovacs *et al.*, 1999; Popowski *et al.*, 2001) because the release of arginine vasopressin (anti diuretic hormone) is increased during exercise, which limits the ability of the kidney to excrete water, thus increasing urine osmolality (Rosner, 2008).

### 2.8.4 Plasma Volume Changes

Plasma volume changes can be calculated from repeated measurements of haematocrit and blood haemoglobin concentration (Dill and Costil, 1974). Due to its relative simplicity, this technique has been extensively utilised in research (Armstrong, 2005). However, plasma volume is subject to variations in posture, food and fluid intake (Maughan and Shirreffs, 2010). According to Johansen *et al.* (1998) acute postural changes can lead to an underestimation of the changes in plasma volume up to 50%; however, this can be minimised if the measurements are taken in a similar posture every time.

All the hydration assessments described have methodological limitations; therefore, the use of more than one technique (laboratory- and field-based) is recommended to obtain a better indication of hydration status.

## 2.9 Summary

The use of carbohydrate beverages (sport drinks) before and during exercise is common practice among endurance and team sports athletes in order to postpone the effects of fatigue. However, there is great variation in the composition and the performance outcomes produced by the different types of sports drinks. The most utilised beverage composition during intermittent high-intensity exercise is 6 to 7% of carbohydrates, mainly glucose or glucose polymers and electrolytes. However, several studies have reported higher rates of carbohydrate oxidation when a mix of multiple transportable carbohydrates (glucose and fructose or maltodextrin and fructose) was ingested (Jentjens *et al.*, 2004; Wallis *et al.*, 2005). The ingestion of caffeine has been also related with exercise improvements due to blockage of adenosine receptors and the delay of the feelings of exertion during exercise. Additionally, caffeine may increase the rate of fluid absorption in the small intestine and therefore increase the rate of carbohydrate oxidation.

Therefore, the study aims were to evaluate the effects of the combined ingestion of a higher dose of carbohydrates (10% maltodextrin and fructose, 2:1) with caffeine (3.2 $mg \cdot kg^{-1} \cdot h^{-1}$) on high-intensity exercise performance and metabolic responses. Additionally, some studies have reported that the ingestion of high doses of carbohydrates (>8%) may reduce gastric emptying and intestinal fluid absorption (Vist and Maughan, 1994; Gisolfi *et al.*, 1992). These responses may aggravate the exercise-induce dehydration. Therefore, several field and laboratory methods were used to assess hydration status under the different treatment conditions.

# Chapter 3

## Methods

## 3.0 Methods

### 3.1 Participants' Characteristics

Fourteen recreational soccer players (males) regularly involved in training and competition (at least twice a week) volunteered to take part in the study (participants' characteristics are presented in Table 3.1). After the study was approved by a University Ethics Committee (Appendix A), all participants received information about the nature and possible risks associated with the study (Appendix B). Written informed consent to take part in the study was obtained from each player (Appendix C). Players were recruited on the basis that they had no injuries, were non- diabetic, were not smokers or had a history of cardiovascular problems (Appendix D).

**Table 3.1** Summary of the participants' characteristics ($n$ = 14).

| Characteristics | mean ± *SEM* |
|---|---|
| **Age (years)** | 24 ± 1 |
| **Mass (kg)** | 79.1 ± 2.3 |
| **Height (m)** | 1.80 ± 0.02 |
| **Estimated maximal oxygen uptake ($mL \cdot kg^{-1} \cdot min^{-1}$)** | 48.7 ± 1.6 |

### 3.2 Preliminary Sessions

Participants reported to the laboratory on two separate occasions before completing the experimental trials. During the first visit, maximum oxygen uptake ($\dot{V}O_{2\ max}$) was estimated using the Multistage Fitness Test (MSFT) and the associated regression equation (Ramsbottom *et al.*, 1988). During the MSFT, players were required to complete 20-m shuttles at a progressive pace until exhaustion. The protocol began at a speed of 2.22 $m \cdot s^{-1}$ with increases of 0.14 $m \cdot s^{-1}$ every minute. The level achieved during the test (speed achieved) was then used to estimate $\dot{V}O_{2\ max}$ using the associated

regression equation (Ramsbottom *et al.*, 1988). These values were used to determine the 40% and 85% $\dot{V}O_{2\,max}$ running speeds of the soccer match simulation protocol (SMS).

During the second preliminary session, participants performed 10 isolated dribbles, 10 isolated passes and 45 min of the SMS (passes and dribbles included), in order to habitualise the participants with the experimental protocol, and to minimise anticipated trial order effects. The layout of the dribbling and passing activities is described in Section 3.4.3.2 and Section 3.4.3.3.

### 3.3 Study Design

Participants completed three experimental trials separated by at least 5 days. The study used a double-blind repeated-measures design where participants were allocated in a balance randomised fashion to receive either a 10% maltodextrin plus fructose (2:1) drink with 29.4 mg of caffeine per 100 ml of solution (CHO10), a 6% maltodextrin plus fructose (2:1) drink (CHO6), and a flavoured placebo drink (PLA). The composition of the drinks is presented in Table 3.2. Additionally, participants ingested 4×60 ml treatment gels (CHO active and placebo) during the trials. The active gels were provided with the carbohydrate drinks (6% CHO and 10% CHO), and the placebo gels were provided with the placebo drink. Two gels were ingested 30 min before the start of the SMS protocol and two during the half-time period. The treatment drinks and gels were specifically manufactured for the study (High Five Ltd., UK) matching appearance, texture and taste. The nutritional and energy composition of the drinks and gels described in Table 3.2 were independently analysed by an external laboratory (Eclipse Scientific Group, UK). Drinks were provided in opaque drinking bottles and the gels were manufactured with identical wrappings so the participants were unable to identify the treatment order.

**Table 3.2** Composition of the treatment beverages and gels (mean ± *SEM*).

| | Drinks | | | Gels | |
|---|---|---|---|---|---|
| | **PLA (per 100 ml)** | **CHO6 (per 100 ml)** | **CHO10 (per 100 ml)** | **Active (per 100 ml)** | **Placebo (per 100 ml)** |
| **Solution Osmolality (mosm·kg$^{-1}$)** | 77.5 ± 0.5 | 112 ± 15.5 | 292 ± 3 | 1249.5 ± 30.5 | 81.5 ± 8.5 |
| **Caffeine (mg)** | 0 | 0 | 29.4 | 0 | 0 |
| **Fat (g)** | 0 | 0 | 0 | 0.3 | 0.3 |
| **Protein (g)** | 0 | 0 | 0 | 0.15 | 0.15 |
| **Carbohydrate (g)** | 0.5 | 5.6 | 9.4 | 37.3 | 0.4 |
| **Energy (kJ/kcal)** | 8.4/2 | 99.2/23.7 | 176.7/42.2 | 647/152 | 20.1/5 |
| **Potassium (mg)** | 25.2 | 19.3 | 20.9 | 21.5 | 19.2 |
| **Magnesium (mg)** | 0.6 | 0.1 | 0.1 | 1.0 | 1.3 |
| **Sodium (mg)** | 83.2 | 78 | 79.5 | 47.3 | 50.1 |

### 3.3.1 Ingestion Rates

Participants consumed a bolus of 5.25 $ml \cdot kg^{-1}$ BM of the allocated treatment drink along with two treatment gel sachets (2 × 60 ml) 30 min before the start of the SMS protocol and during the half-time recovery period. This equated to a mean volume of 831 ± 14 ml of the treatment drink. Additionally, participants consumed 2.63 $ml \cdot kg^{-1}$ BM of the treatment solution at 15 and 30 min in each half of the SMS. This equated to a mean volume of 415 ± 7 ml during each half of the SMS protocol.

During the SMS protocol (135 min), treatment drinks were ingested at a rate of 21 $ml \cdot kg^{-1}$ BM (9.3 $ml \cdot kg^{-1} \cdot hr^{-1}$). In total, participants ingested an average of 1661 ± 27 ml, plus 240 ml of treatment gels during each main trial (breakfast drink not included). Participants consumed an average of 246 g of carbohydrate (1.8 $g \cdot min^{-1}$) in the CHO10 trial, 183 g of carbohydrate (1.3 $g \cdot min^{-1}$) in the CHO6 trial and 9 g of carbohydrate (0.07 $g \cdot min^{-1}$) in the PLA trial. Participants ingested 294 $mg \cdot l^{-1}$ of caffeine in the CHO10 trial, which equals to a rate of 6.1 $mg \cdot kg^{-1}$ of BM. Therefore, participants ingested an average of 488 mg of caffeine during the trials in the CHO10 treatment.

## 3.4 Main Trial Procedures

### 3.4.1 Environmental Conditions

All the experimental trials were performed at the same time of the day to reduce the influence of circadian rhythms on the physiological responses. All trials were performed in an indoor sport facility (indoor athletic track), on a flat synthetic surface. The temperature and humidity in the indoor facility were measured with a digital thermo-hygrometer (iROX ETHG912; Oregon Scientific Ltd., UK) at the beginning and the end of the SMS protocol. The barometric pressure was recorded with a mercury barometer (model 230-7411; Novalynx Corporation, USA) every morning.

### 4.4.2 Nutritional Conditions

Participants were asked to refrain from strenuous physical activity and to avoid the ingestion of alcohol and caffeine for at least 24 h before the main trials. Additionally, participants were asked to record all foods and drinks ingested the day before the main trials and to replicate the food and fluid intake before each main trial (Appendix E). Dietary records were checked prior to each trial to ensure that the instructions had been followed and were later analysed using commercially available software for nutritional analysis (CompEat version 5.8.8; Nutrition Systems, UK).

### 3.4.3 Main Trials

Participants reported to the laboratory at 8:00 am after an overnight fast. Upon arrival, participants voided their bowels and provided a mid-flow urine sample for analysis. Subsequently, body mass (model 770; Seca, Germany) and stature were determined (Harpenden stadiometer; Holtain, UK). A base-line capillary blood sample was drawn before a standardised 1830 kJ breakfast meal (3 slices of white bread, 20 g of margarine, 20 g of marmalade; 55% carbohydrate, 37% fat and 8% protein) was provided along with 500 ml of the treatment drink. Participants then remained in a rested state for approximately 100 min. After the resting period, a capillary blood sample (pre-SMS) was taken. Subsequently, participants were taken to an adjacent indoor sport facility to complete the SMS protocol (study timeline is presented in Figure 3.1). Immediately after the completion of the SMS protocol, body mass was measured and a capillary blood sample was taken (post-SMS), followed by a 5-min cool-down jog.

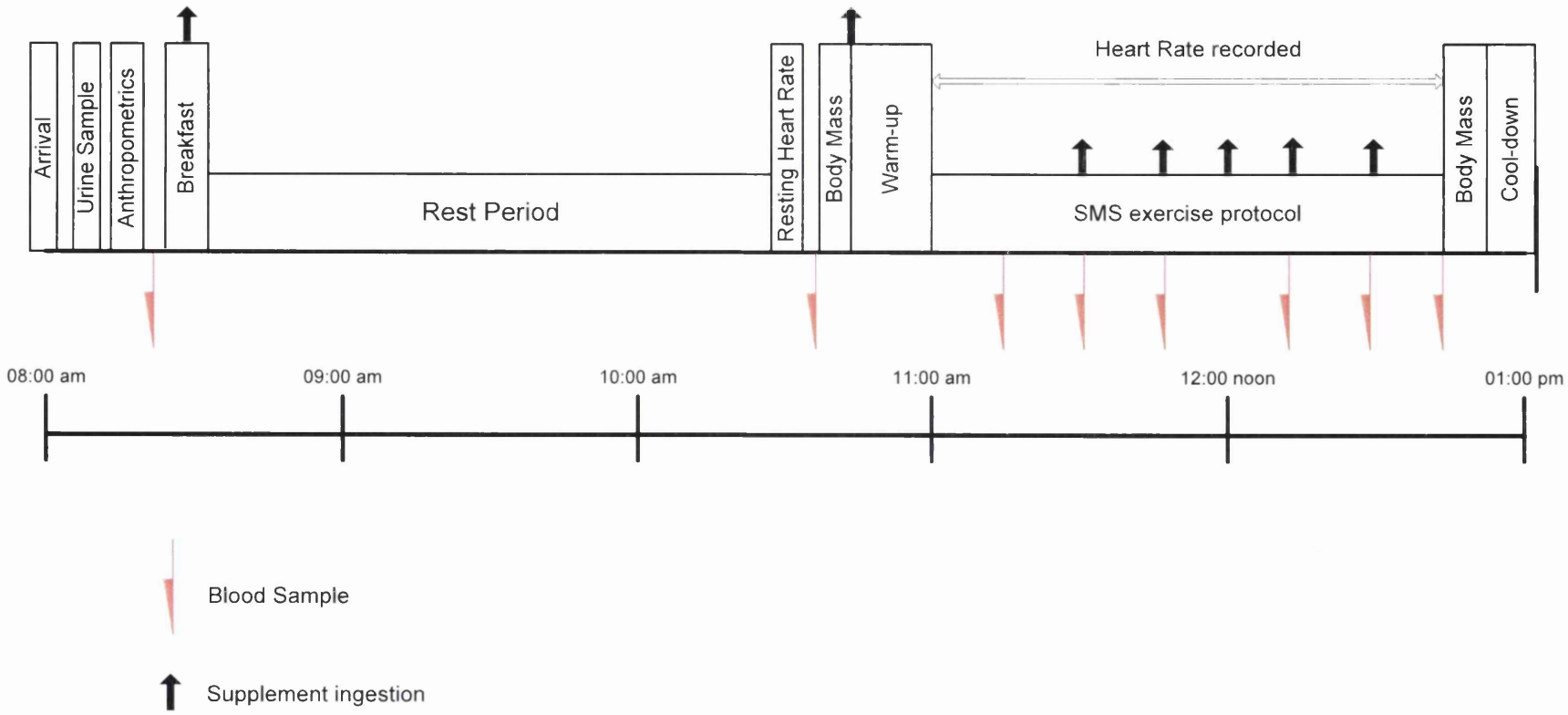


**Figure 3.1** Schematic time-line of the main trial procedures.

### 3.4.3.1 The Soccer Match Simulation (SMS)

Upon arrival to testing venue, body mass (pre-SMS) was measured and a fluid bolus of the allocated drink (5.25 ml · $kg^{-1}$ BM) and two gel sachets (2 × 60 ml) were provided. After the ingestion of the supplements, participants performed a 20-min standardised warm-up (Appendix F).

Immediately after the warm-up, participants commenced the soccer match simulation protocol (SMS). The soccer match simulation (SMS) protocol was similar to the one devised by Nicholas *et al.* (2000), with additional components added that further replicated the patterns and movement demands of soccer match-play (Russell *et al.*, 2011a). A schematic of the SMS protocol layout is presented in Figure 3.2. Participants completed two 45-min halves of soccer-specific activities separated by a 15-min passive recovery period (half time). Each 45-min halves included six 5.5-min soccer activity blocks separated by a 2-min passive recovery. Moreover, each 5.5-min soccer activity block incorporated 3 repeated exercise cycles and a 1-min passing activity. Each exercise cycle was made up of three 20-m walks (2.2 $m \cdot s^{-1}$), one alternated 15-m sprint or 20-m ball dribble, five 20-m jogs (40% $\dot{V}O_{2\,max}$), one 20-m backwards jog (40% $\dot{V}O_{2\,max}$) and two 20-m runs (85% $\dot{V}O_{2\,max}$).

The walking, jogging and running movement speeds were dictated by audio signals emitted by a CD player. Sprint times were recorded using timing gates (Brower Timing Gates Ltd., USA) and used to calculate sprint speeds. The speed values were averaged in 15 min blocks and used as a high-intensity exercise performance marker. During the SMS protocol, participants covered a total distance of 10 km and performed eighteen 15-m sprints, eighteen dribbles and forty-eight passes.

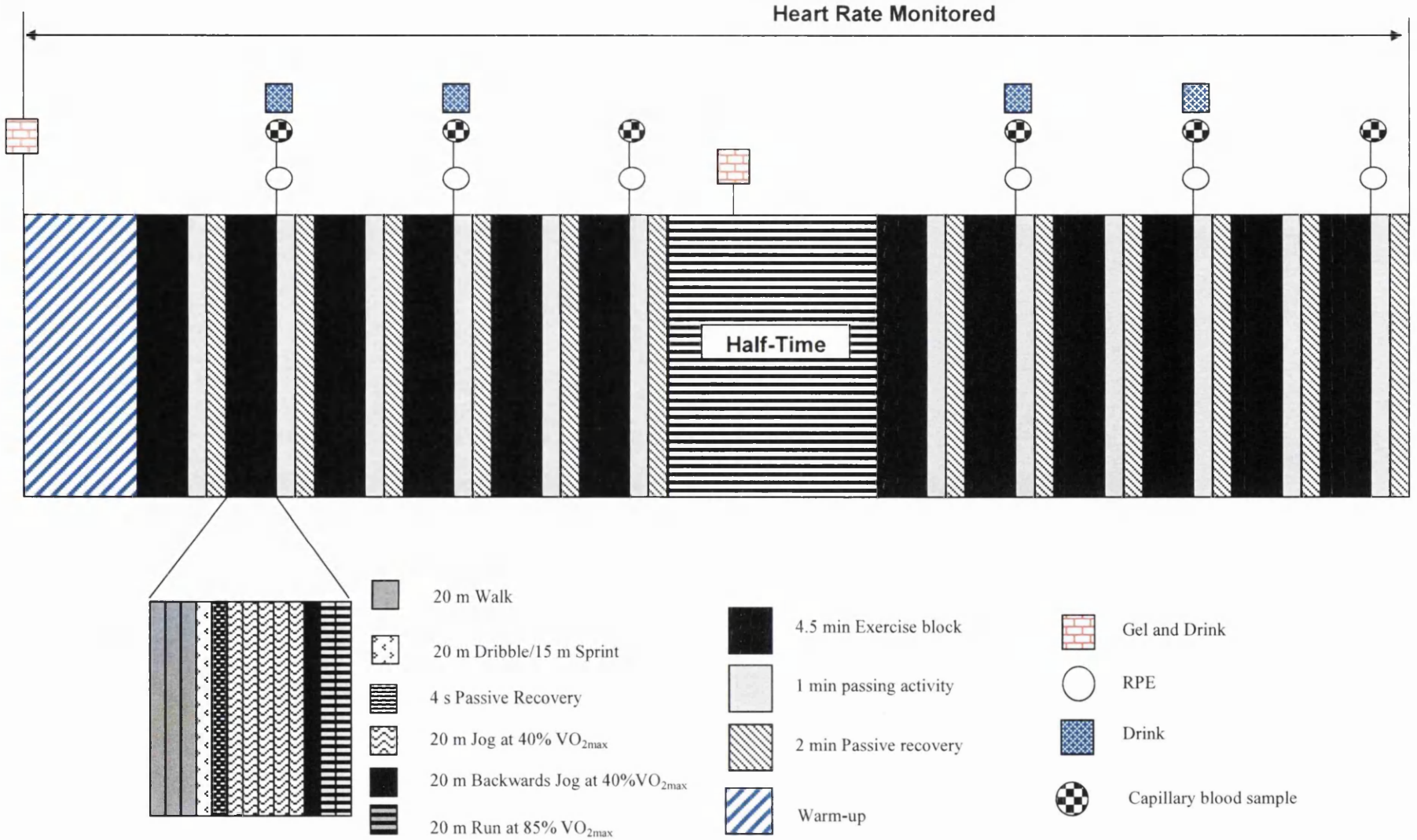


**Figure 3.2** Schematic of the soccer match simulation protocol.

### 3.4.3.2 Dribbling Activity

The dribbling activity was identical to that employed by Russell *et al.* (2010; 2011a), with start and finish lines placed 20 m apart. Cones 2 through 7 were placed 3 m away from the preceding cone and cone 1 and 7 were 1 m away from the starting and finish lines (Figure 3.3). Participants were instructed to drive the ball (controlled) as fast as possible around the cones.

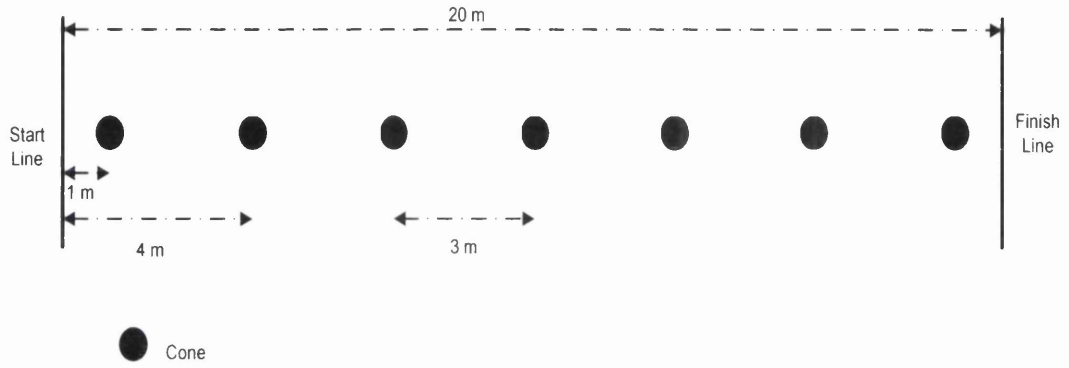


**Figure 3.3** Schematic diagram of the ball dribbling activity.

### 3.4.3.3 Passing Activity

The layout of the passing activity was similar to the one described by Russell *et al.* (2011a). However, only two passing targets were used as it was believed that the passing movement patterns were accurately represented using only two passing targets. According to Russell *et al.* (2011a), the layout of the passing activity replicated the soccer passing motor skill performance and the visual search and decision making performed by soccer players during game situations (a schematic of the passing layout is presented in Figure 3.4).

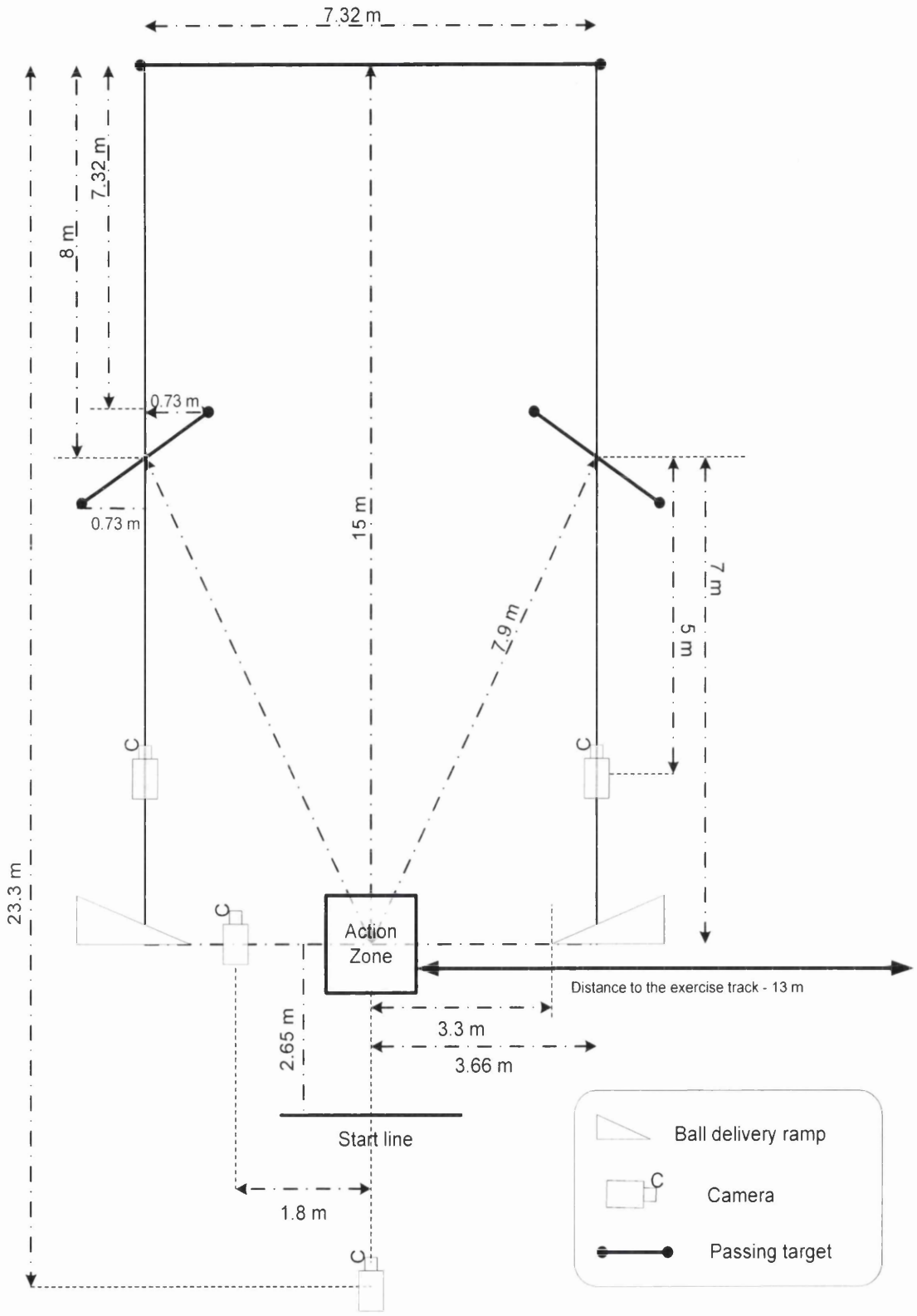


**Figure 3.4** Schematic of the passing activity layout.

### 3.5 Anthropometric Assessments

Anthropometric measurements were performed according to the procedures described by the International Society for the Advancement of Kinanthropometry (ISAK). Stature was measured using a portable stadiometer (Harpenden Portable Stadiometer; Holtain Ltd., UK) to the nearest 0.1 cm.

#### 3.5.1 Body Mass Changes

Body mass was measured (participants in minimal clothing) with a digital scale to the nearest 0.1 kg (Seca 710; Seca, Germany). Participants were weighed wearing only underpants before and after the SMS protocol. Between these two weighing periods, all fluids ingested and all urine excreted was recorded. Sweat losses were estimated by correcting the changes in body mass for fluid ingested and urine passed. Post-SMS body mass was measured after participants towelled off the excess of sweat from their bodies.

During exercise not longer than 4 hours, it can be generally assumed that the acute changes in body mass over a short period of time are due to body water loss or gain (Oppliger and Bartok, 2002; Shirreffs, 2003). Changes in body mass were used to determine the volume of sweat loss and sweat rates during the SMS protocol (Equation 3.1 and 3.2). Water loss due to respiration was ignored in the calculation and was included as sweat volume. Body mass gains due to substrate metabolism were also ignored in the calculations as the volume of metabolic water produced during cellular metabolism (~0.13 $g \cdot kcal^{-1}$) is approximately equal to respiratory water losses (~0.13 $g \cdot kcal^{-1}$) (Mitchell *et al.*, 1972).

$$\textit{Sweat loss (l)} = \textit{(preBM} - \textit{postBM)} - \textit{(UO)} + \textit{(FI)}$$

**Equation 3.1** Equation to calculate sweat loss (l), where preBM: pre-exercise body mass (kg), postBM: post-exercise body mass (kg), FI: fluid intake (l), and UO: urine output (l).

*Sweat rate ($l \cdot h^{-1}$) = (water loss / exercise time) · 60*

**Equation 3.2** Equation to calculate the rate of sweat loss ($l \cdot h^{-1}$).

## 3.6 Hydration Assessments

### 3.6.1 Urine and Plasma Osmolality

Urine osmolality was measured on arrival to the laboratory to evaluate initial hydration status. Urine osmolality was determined (in duplicate) by freezing point depression (Gonotec Cryoscopic Osmometer 030; YSI Ltd., UK). The intra-assay co-efficient of variance (CV) of the determination of urine osmolality was 1.0%.

Plasma osmolality was measure by freezing point depression (Gonotec Cryoscopic Osmometer 030; YSI Ltd., UK) before and after the SMS protocol. An aliquot (~210 µl) of whole blood was centrifuged at 1735 g for 15 min (Heraeous Labofuge 400R; DJB Labcare Ltd., UK) to separate plasma and the haematocrit portion. The plasma osmolality was then measured. The intra-assay CV of the determination of plasma osmolality with the Osmomat 030 was 1.25%.

### 3.6.2 Blood Haemoglobin

Blood haemoglobin was determined (in duplicate) using an automated 2-wavelength photometer (B-Haemoglobin analyser; HemoCue Ltd., UK). Blood haemoglobin was determined from all capillary blood samples taken during the main trials. The intra-assay CV of the haemoglobin determination with the B-Haemoglobin analyser was 4.2%.

### 3.6.3 Blood Haematocrit

Blood haematocrit was determined using an automated blood analyser (GEM Premier 3000; Instrumentation Laboratory Ltd., UK) by an electrical conductivity technique. The conductivity technique is based on the principle that because plasma is more conductive than blood cells due to the high resistance of the cell membranes, the resistivity of blood will increase as the concentration of cells increases. Blood haematocrit using the GEM Premier 3000 was determined in all the capillary samples taken during the main trials. The CV of the GEM Premier 3000 haematocrit determination was 3%.

To validate the automated method of blood haematocrit analysis (GEM Premier 3000), blood haematocrit was also determined using the micro-centrifuged method pre and post-SMS protocol (Pearson correlation: $r = 0.61$; $P < 0.0001$) (Appendix J). An aliquot of capillary whole blood was collected into heparinised capillary tubes (80 µL tubes; Hawksley and Sons Ltd., UK). The tubes were sealed with clay and spun at 13000 g for 5 min (Micro-haematocrit centrifuge; Hawksley and Sons, UK). A manual micro-haematocrit reader (Hawksley Reader; Hawksley and Sons, UK) was then used to quantify the haematocrit percentage in the sample. The haematocrit result was averaged and corrected for the trapped plasma that remains in the red blood cell portion (Johansen *et al.,* 1998). The CV of the micro-haematocrit determination was 1.6%.

### 3.6.4 Plasma Volume Changes

Measurements of blood haemoglobin and haematocrit concentration have been widely used to represent changes in plasma volume (Dill and Costil, 1974; Harrison, 1985; Linnane *et al.*, 2004; Bishop and Maxwell, 2009). Changes in plasma volume were calculated using the blood haemoglobin and haematocrit values using the Dill and Costill (1974) method. Postural changes from lying to standing can result in a decrease in plasma volume of up to 10% (Rowel, 1974, Harrison, 1985). Therefore, all blood samples during the main trials were taken from a standardised standing position.

### 3.7 Capillary Blood Sampling

Capillary blood samples were taken throughout the main trials to assess the metabolic responses and the hydration status of individuals. Capillary blood samples were drawn from the finger tip. The finger surface area was cleaned with alcohol swabs and left to dry. Subsequently, the skin was punctured with a disposable lancet (Accu-Check Safe-T-lancets; Roche Products Ltd., UK) and the first drop of blood was removed before collecting whole blood into heparinised capillary tubes (170 μL tubes; Instrumentation Laboratory Ltd., UK and 80 μL tubes; Hawksley and Sons Ltd., UK) for analysis.

Approximately 380 μl of whole blood was drawn pre and post-SMS protocol (1 × 170 μl tube and 3 × 80 μl tubes). A volume of ~150 μl (170 μl tube) of whole blood was used for metabolites and electrolytes analysis (GEM Premier 3000; Instrumentation Laboratory Ltd., UK). A volume of ~210 μl (3 × 80 μl tubes) of whole blood was centrifuged at 1735 g for 15 min (Labofuge 400R; Kendro Lab Products, Germany) to be separated into its components. Subsequently, from the centrifuged sample, a volume of ~50 μl of plasma was used to determine plasma osmolality by freezing point depression (Gonotec Cryoscopic Osmometer 030; YSI Ltd., UK).

In addition to the pre and post-SMS capillary blood samples, five samples were drawn during the SMS protocol at 15, 30, 45, 60 and 75 min and analysed for metabolites, electrolytes and haematocrit (GEM Premier 3000; Instrumentation Laboratory Ltd., UK).

### 3.8 Determination of Blood Glucose, Lactate and Sodium ($Na^+$) concentrations

The blood concentrations of glucose, lactate, and $Na^+$ were determined using the GEM Premier 3000 (Instrumentation Laboratory Ltd., UK). The central component of the GEM Premier 3000 is the reagent cartridge (iQM cartridge 24345089; Instrumentation Laboratory Ltd., UK), which contains the analytical sensors, flow system, calibrators, process control modules, wash solution and waste receptacle. The $Na^+$, glucose, lactate

and haematocrit sensors, together with the reference electrode, are integral parts of the chamber, with chemically sensitive membranes permanently bonded to the chamber body (Figure 3.5).

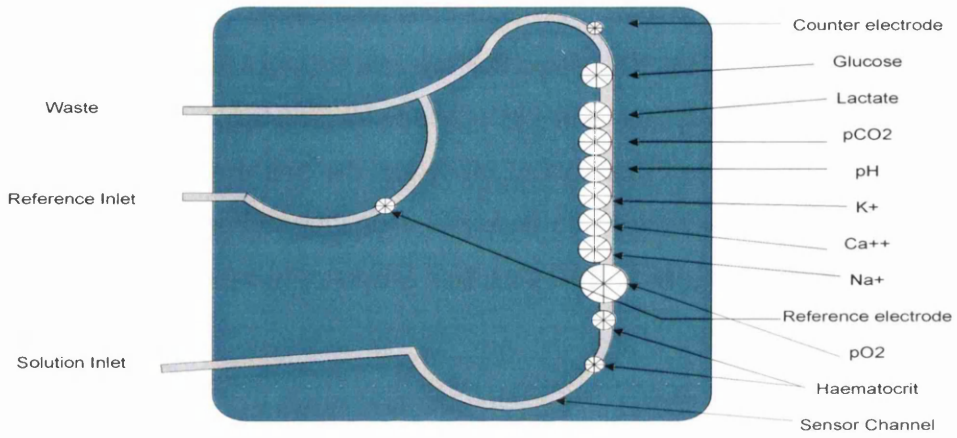


**Figure 3.5** Schematic of GEM 3000 sensor card and miniaturised sensors (Fallon *et al.*, 2003).

Glucose and lactate were determined by enzymatic reaction with oxygen in the presence of glucose oxidase or lactate oxidase to produce hydrogen peroxide ($H_2O_2$). Electrolytes ($Na^+$) were measured with polyvinyl chloride sensors based on the principle of ion-selective electrodes; that is, an electrical potential can be established across a membrane which selectively binds to a specific ion. The CV were below 3% for all the variables (10 samples), except for the lactate analysis (CV = 12%).

### 3.9 Rate of Perceived Exertion

Ratings of perceived exertion (Borg, 1973) were recorded immediately after each block of exercise during the SMS protocol. Participants rated their exertion levels on a visual scale (6-20), with 6 representing the lowest and 20 the highest degree of exertion (Appendix G).

### 3.10 Statistical Analysis

Statistical analysis was performed using SPSS software (version 16.0; SPSS Inc., USA). The statistical significance was set at $P \leq 0.05$ and all results were reported as the mean ± standard error of the mean (*SEM*). The coefficient of variation (CV) was calculated from at least 15 random sample from each assay ((SD/mean)*100). Bland and Altman plot was utilised to establish the level of agreement between the automated device (GEM 3000) and the micro-centrifuged haematocrit assessments. One-way repeated measures analyses of variance (ANOVA) were used where data contained a single time point. Two-way repeated measures ANOVA (within-subject factors: trial × time of sample) were conducted where the data included multiple sample time points. Mauchly's test was consulted and Greenhouse-Geisser correction was applied if the assumption of sphericity was violated. If a significant *P*-value was identified for the interaction effect (trial × time), it was considered that trial influenced the response over the exercise protocol and simple main effects analyses were performed. If significant main effects of time or trial were identified further investigation was carried out using single-step multiple comparison Tukey post hoc analyses.

# Chapter 4

## Results

## 4.0 Results

### 4.1 Dietary Intake

The macronutrients content and energy intakes were similar between trials (Table 4.1). Overall, the mean caloric intake prior the main trails was 7070 ± 466 kJ, where 47.6 ± 2% were carbohydrates, 34.6 ± 2% fats and 17 ± 1% proteins.

**Table 4.1** Dietary intake prior each trial ($n$ = 14).

| | Trials | | | |
|---|---|---|---|---|
| | **PLA** | **CHO6** | **CHO10** | ***P* value** |
| **Energy (kJ)** | 6904 ± 452 | 7277 ± 473 | 7029 ± 473 | 0.810 |
| **Protein (g)** | 66.7 ± 4.9 | 79.5 ± 5.0 | 71.3 ± 6.3 | 0.137 |
| **Fat, Total (g)** | 69.6 ± 5.1 | 69.8 ± 5.4 | 64.5 ± 3.9 | 0.679 |
| **Carbohydrate (g)** | 201.8 ± 18.1 | 206.6 ± 14.5 | 216.1 ± 21.2 | 0.836 |
| **Sugars, Total (g)** | 67.6 ± 16.6 | 74.2 ± 13.0 | 72.2 ± 11.3 | 0.822 |
| **Starch (g)** | 132.6 ± 11.8 | 128.6 ± 7.4 | 138.7 ± 114.3 | 0.833 |
| **Fibre (g)** | 9.6 ± 0.9 | 10.0 ± 0.9 | 7.9 ± 0.6 | 0.199 |
| **Calcium (mg)** | 657.5 ± 66.6 | 668.6 ± 102.6 | 711.7 ± 87.9 | 0.902 |
| **Sodium (mg)** | 2619 ± 208 | 2297 ± 250 | 2297 ± 192 | 0.577 |
| **Thiamine (B1) (mg)** | 1.1 ± 0.1 | 1.1 ± 0.1 | 1.2 ± 0.1 | 0.806 |
| **Riboflavin (B2) (mg)** | 1.0 ± 0.1 | 1.2 ± 0.1 | 1.3 ± 0.2 | 0.576 |
| **Vitamin B6 (mg)** | 1.3 ± 0.2 | 1.8 ± 0.1 | 1.7 ± 0.3 | 0.264 |
| **Vitamin B12 (µg)** | 3.0 ± 0.6 | 3.9 ± 0.4 | 4.5 ± 1.0 | 0.171 |
| **Folates (µg)** | 177 ± 30 | 164 ± 21 | 173 ± 16 | 0.906 |
| **Vitamin C (mg)** | 62.4 ± 21.2 | 65.0 ± 21.1 | 56.1 ± 17.9 | 0.945 |
| **Vitamin A (µg)** | 405 ± 88 | 350 ± 71 | 304 ± 62 | 0.671 |
| **Vitamin D (µg)** | 1.4 ± 0.4 | 1.7 ± 0.3 | 3.0 ± 1.8 | 0.383 |
| **Vitamin E (mg)** | 6.0 ± 0.6 | 4.9 ± 0.7 | 5.2 ± 1.1 | 0.682 |
| **Cholesterol (mg)** | 231 ± 49 | 225 ± 21 | 204 ± 39 | 0.878 |
| **Alcohol (g)** | 0.9 ± 0.9 | 1.6 ± 1.6 | 0.5 ± 0.5 | 0.608 |
| **Water (ml)** | 1501 ± 253 | 1751 ± 251 | 1870 ± 278 | 0.340 |

Values represent the mean ± *SEM*, $n$ = 14. $P$ value represents trial effect.

## 4.2 Environmental Data

The ambient temperatures were similar during all trials (CHO10: 18.7 ± 0.4 °C, CHO6: 18.6 ± 0.1 °C, PLA: 19.0 ± 0.2 °C; trial effect: $F_{(2, 26)} = 0.50$, $P = 0.61$, $\eta_p^2 = 0.04$). Similarly, relative humidity (CHO10: 59 ± 2%, CHO6: 62 ± 2%, PLA: 63 ± 2%; trial effect: $F_{(2, 26)} = 0.71$, $P = 0.50$, $\eta_p^2 = 0.05$), and atmospheric pressure (CHO10: 1015 ± 2 hPa, CHO6: 1014 ± 2 hPa, PLA: 1015 ± 2 hPa; trial effect: $F_{(2, 26)} = 0.12$, $P = 0.89$, $\eta_p^2 = 0.01$) did not differ between trials.

## 4.3 Metabolic Responses

### 4.3.1 Blood Glucose Concentration

The pattern of response in blood glucose concentrations was influenced by supplementation during the SMS protocol (trial × time interaction effect: $F_{(12, 156)} = 8.14$, $P < 0.001$, $\eta_p^2 = 0.39$). Post hot tests revealed that blood glucose concentrations were 17.6%, 18.7%, 29.8%, 12.9% and 26.8% higher in the CHO10 trial when compared with the PLA trial at 15, 30, 45, 75 min and post-SMS respectively ($P < 0.05$). Additionally, blood glucose concentrations were 13.3% and 16% higher in the CHO10 trial when compared with the CHO6 trial at 45 and 75 min respectively ($P < 0.05$). Blood glucose concentrations were 19% and 17.4% higher in the CHO6 trial when compared with the PLA trial at 45 min and post-SMS protocol ($P < 0.05$).

Post hoc tests also revealed that blood glucose concentrations were affected throughout the SMS protocol. Blood glucose concentrations decreased after the half-time recovery period (45min to 60 min) by 40%, 38.4% and 16% in the CHO10, CHO6 and PLA trials respectively ($P < 0.05$). Blood glucose concentrations were still 29.1%, 31.3% and 12% lower at 75 min when compared with the 45 min sample in the CHO10, CHO6 and PLA trials respectively ($P < 0.05$) (Figure 4.5).

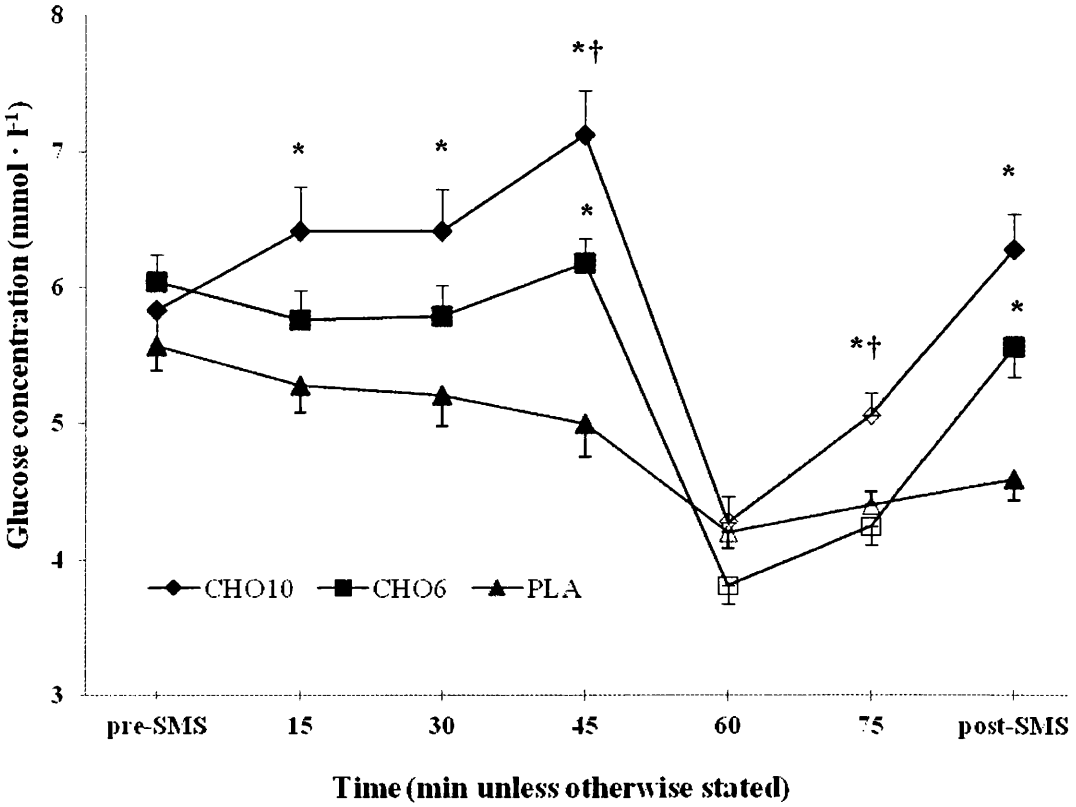


**Figure 4.1** Blood glucose concentrations measured during the SMS protocol. Values represent the mean ± *SEM*, $n = 14$. * symbol indicates higher blood glucose concentrations than PLA ($P< 0.05$). † symbol indicates higher blood glucose concentrations than CHO6 trial ($P< 0.05$). Open markers (e.g. □, Δ) indicate lower blood glucose concentrations compared with 45 min time point ($P<0.05$).

### 4.3.2 Blood Lactate Concentration

The pattern of response in blood lactate concentrations was affected by supplementation during the SMS protocol (trial × time interaction effect: $F_{(5.4, 70.0)} = 2.54$, $P = 0.03$, $\eta_p^2 = 0.16$). Post hoc tests revealed that blood lactate concentrations were higher in the CHO10 trial when compared with the PLA trial at 60 min (CHO10: 7.2 ± 0.7 mmol·l$^{-1}$, PLA: 6.0 ± 0.9 mmol·l$^{-1}$) and 75 min (CHO10: 7.5 ± 0.7 mmol·l$^{-1}$, PLA: 6.0 ± 0.9 mmol·l$^{-1}$, $P<0.05$). Additionally, lactate concentrations were higher in the CHO10 trial when compared with the CHO6 trial at 60 min (CHO6: 5.9 ± 0.7 mmol·l$^{-1}$), 75 min (CHO6: 5.1 ± 0.8 mmol·l$^{-1}$) and post-SMS protocol (CHO10: 7.4 ± 0.8 mmol·l$^{-1}$, CHO6: 5.6 ± 0.8 mmol·l$^{-1}$, $P<0.05$) (Figure 4.4).

Blood lactate was also influenced by the exercise protocol. Post hoc tests revealed that blood lactate concentrations were higher in the CHO6 and PLA trials at 15 min when compared with samples taken during the second half of the SMS protocol (60, 75 min and post-SMS, $P < 0.05$).

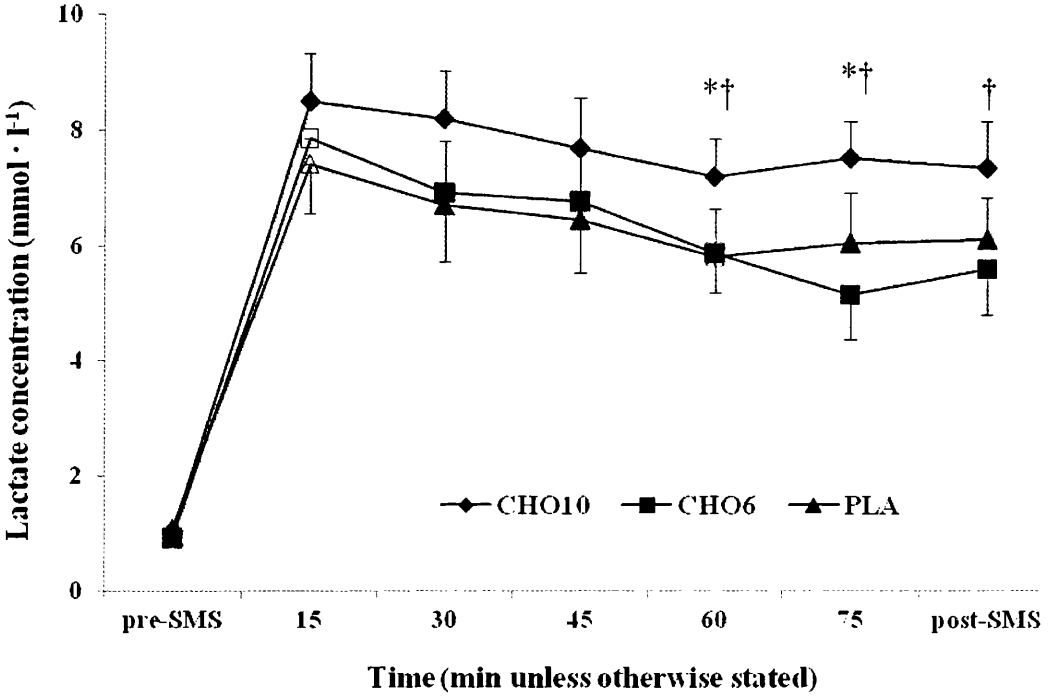


**Figure 4.2** Blood lactate concentrations measured during the SMS protocol. Values represent the mean ± *SEM*, $n = 14$. * symbol indicates higher lactate concentrations than PLA ($P<0.05$). † symbol indicates higher lactate concentrations than CHO6 ($P<0.05$). Open markers (e.g. □, Δ) indicate significantly higher lactate concentration compared with the 60, 75 min and post-SMS samples ($P < 0.05$).

## 4.4 Hydration Indices

### 4.4.1 Urine and Plasma Osmolality

Participants arrived in a similar state with similar urine osmolalities across trials (CHO10: 842 ± 53 mosm·l$^{-1}$, CHO6: 722 ± 66 mosm·l$^{-1}$, PLA: 799 ± 48 mosm·l$^{-1}$; trial effect: $F_{(2, 26)} = 1.57$, $P = 0.22$, $\eta_p^2 = 0.11$). Similarly, pre-exercise (pre-SMS) plasma osmolality was similar between trials (CHO10: 292 ± 3 mosm·l$^{-1}$, CHO6: 291 ± 2 mosm·l$^{-1}$, PLA: 292 ± 3 mosm·l$^{-1}$; trial effect: $F_{(2, 26)} = 0.09$, $P = 0.91$, $\eta_p^2 = 0.01$).

Plasma osmolality followed a similar pattern of response during all trials (trial × time interaction: $F_{(1.4, 18.1)} = 1.14$, $P = 0.34$, $\eta_p^2 = 0.08$). Plasma osmolality was not affected by supplementation (CHO10: 295 ± 3 mosm·l$^{-1}$, CHO6: 293 ± 2 mosm·l$^{-1}$, PLA: 293 ± 3 mosm·l$^{-1}$; trial effect: $F_{(2, 26)} = 0.17$, $P = 0.84$, $\eta_p^2 = 0.01$). However, plasma osmolality was higher post-exercise when compared with the pre-exercise values (time effect: $F_{(1, 13)} = 8.20$, $P = 0.01$, $\eta_p^2 = 0.39$). Post hoc tests revealed that plasma osmolality post-exercise was 2% higher than the pre-SMS value in the CHO10 trial ($P < 0.05$) (Figure 4.1).

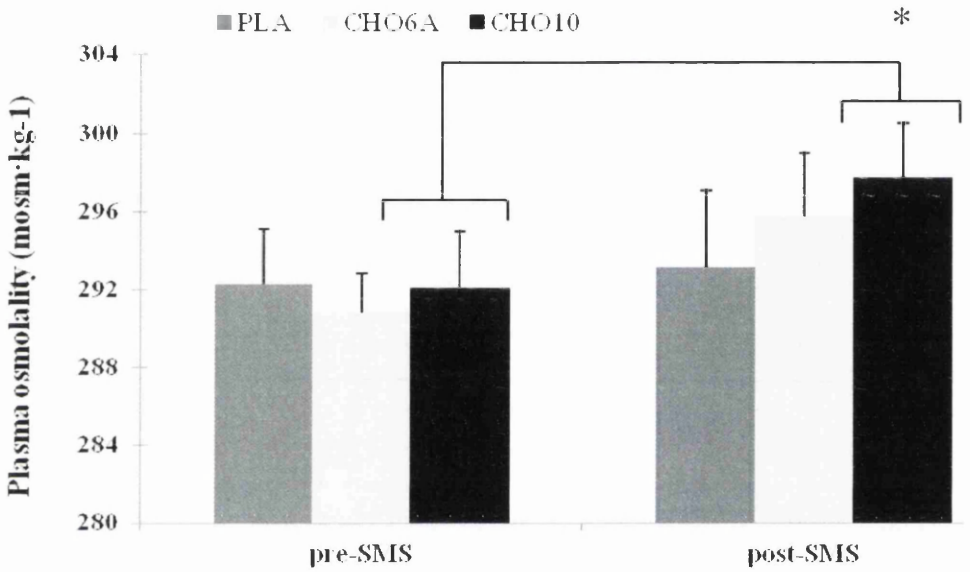


**Figure 4.3** Plasma osmolality responses pre and post-exercise (pre-SMS and post-SMS). Values represent the mean ± *SEM*, $n$ =14. * indicates significantly higher plasma osmolality compared with the Pre-SMS sample ($P< 0.05$).

### 4.4.2 Plasma Volume Changes

The pattern of response for the changes in plasma volume was similar for all trials during the SMS protocol (trial × time interaction effect: $F_{(4.7, 60.9)} = 1.42$, $P = 0.23$, $\eta_p^2 = 0.10$) (Figure 4.2). Plasma volume was not different between trials (trial effect: $F_{(2, 26)} = 2.24$, $P = 0.12$, $\eta_p^2 = 0.14$). However, plasma volume was influenced by the exercise protocol (time effect: $F_{(6, 78)} = 3.91$, $P < 0.01$, $\eta_p^2 = 0.23$). Post hoc tests revealed a reduction in plasma volume in the CHO10 (7.9%) and CHO6 (10.7%) trials between the pre-SMS and the 15-min sampling time ($P < 0.05$) and then remained low throughout

the SMS protocol. Additionally, plasma volume increased in the PLA trial towards the end of the SMS protocol, being 8 % and 9.2% higher at 75 min and post-SMS respectively when compared with the 15 min time point.

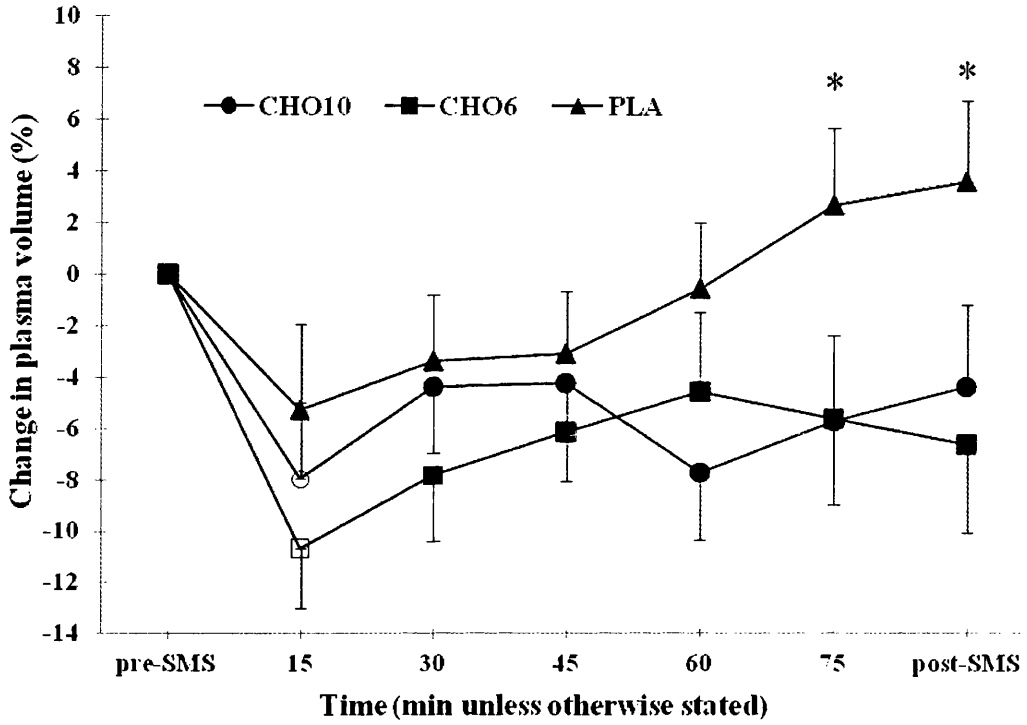


**Figure 4.4** Changes in plasma volume during the SMS protocol. Values represent the mean ± *SEM*, $n = 14$. * symbol indicates higher plasma volume when compared with the 15 min time point. Open markers (e.g. □, Δ) indicate lower plasma volume when compared with the pre-SMS time point ($P< 0.05$).

### 4.4.3 Sodium Concentration

Sodium concentration followed a similar pattern of response across all trials during the SMS protocol (trial × time interaction effect: $F_{(12, 156)} = 1.36$, $P = 0.19$, $\eta_p^2 = 0.09$). Sodium concentrations were influenced by supplementation (trial effect: $F_{(2, 26)} = 7.95$, $P = 0.002$, $\eta_p^2 = 0.38$). Post hoc tests revealed that sodium concentrations were 1.6%, 2.7%, 2.6%, 2.3% and 2.3% higher in the CHO10 trial when compare to the PLA trial at 15, 45, 60, 75 min and post-SMS respectively ($P < 0.05$). Additionally, sodium concentrations were 1.4%, 1.9% and 2% higher in the CHO6 trial when compared with the PLA trial at 15, 60 min and post-SMS respectively ($P < 0.05$) (Figure 4.3).

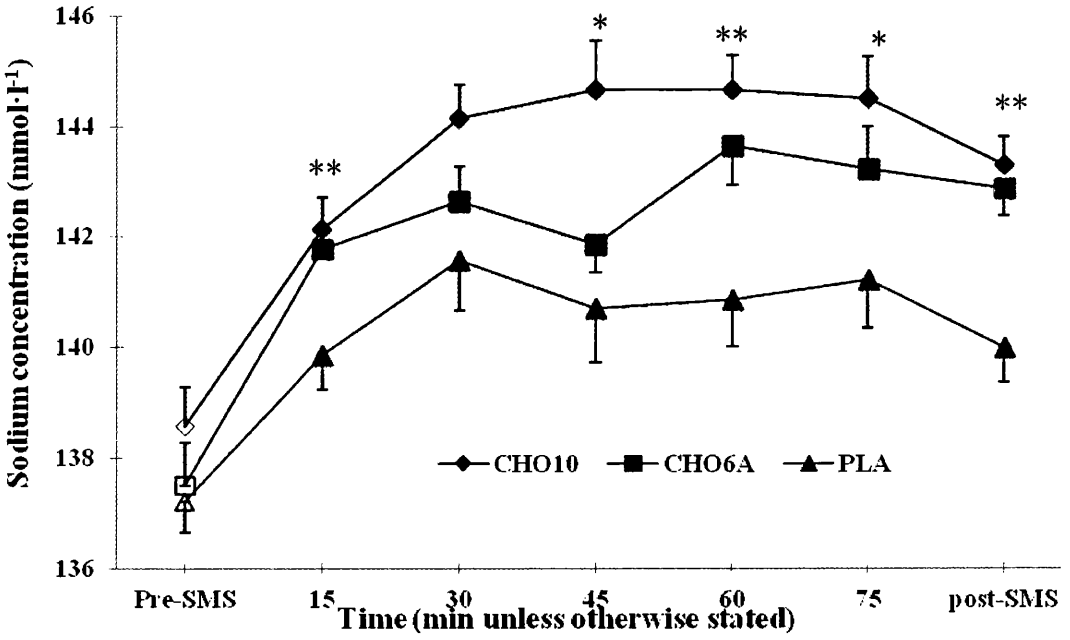


**Figure 4.5** Sodium concentrations during the SMS protocol. Values represent the mean ± *SEM*, $n = 14$. * symbol indicates higher sodium concentrations than PLA ($P < 0.05$). Open markers (e.g. □, Δ) indicate lower sodium concentrations compared with the rest of the time points ($P < 0.05$).

Sodium concentrations were also influenced by the exercise protocol (time effect: $F_{(6, 78)} = 30.77$, $P < 0.001$). Post hoc tests revealed that sodium concentrations were significantly elevated during the SMS protocol (15, 30, 45, 60, 75 and post-SMS) compare with the resting sample (pre-SMS) across all trials ($P < 0.05$).

### 4.5 Body Mass Changes and Sweat Loss

The initial anthropometric characteristics (pre-SMS) were similar between trials, where mean body mass was 79.3 ± 1.3 kg (trial effect: $F_{(2, 26)} = 0.91$, $P = 0.41$, $\eta_p^2 = 0.07$) and mean stature was 1.81 ± 0.01 m (trial effect: $F_{(2, 26)} = 1.10$, $P = 0.34$, $\eta_p^2 = 0.08$) (Table 4.2). Fluid intake was similar between trials (trial effect: $F_{(2, 26)} = 0.11$, $P = 0.89$, $\eta_p^2 = 0.01$), with mean fluid intake being 1.90 ± 0.05 l (including drinks and gels), which equated to an ingestion rate of 0.84 $l \cdot h^{-1}$.

The changes in body mass were similar between trials (CHO10: -0.1 ± 0.1 kg; CHO6: 0.0 ± 0.1 kg; PLA: -0.1 ± 0.1 kg; trial effect: $F_{(2, 26)} = 2.98$, $P = 0.07$, $\eta_p^2 = 0.19$). The amount of sweat loss during the SMS protocol was similar to the volume of fluid ingested and also similar between trials (CHO10: 2.0 ± 0.1 kg, CHO6: 1.9 ± 0.1 kg, PLA: 2.0 ± 0.1 kg; trial effect: $F_{(2, 26)} = 3.1$, $P = 0.06$, $\eta_p^2 = 0.19$) (Table 4.2).

**Table 4.2** Absolute and relative changes in body mass and sweat losses during the SMS protocol.

| | Trials | | |
|---|---|---|---|
| | **PLA** | **CHO6** | **CHO10** |
| **Pre-SMS Body Mass (kg)** | 79.5 ± 2.3 | 79.1 ± 2.2 | 79.3 ± 2.3 |
| **Post-SMS Body Mass (kg)** | 79.4 ± 2.3 | 79.1 ± 2.2 | 79.2 ± 2.3 |
| **Mean Fluid Intake (l)** | 1.90 ± 0.05 | 1.90 ± 0.05 | 1.90 ± 0.05 |
| **Mean Sweat Loss (kg)** | 2.0 ± 0.1 (1.2-2.6) | 1.9 ± 0.1 (1.2-2.4) | 2.0 ± 0.1 (1.3-2.5) |
| **Sweat Rate ($l \cdot h^{-1}$)** | 0.9 ± 0.0 (0.5-1.1) | 0.8 ± 0.0 (0.5-1.1) | 0.9 ± 0.0 (0.6-1.1) |

Values represent mean ± *SEM*, $n$ =14.

### 4.6 Heart Rate and Ratings of Perceived Exertion

Mean heart rate was influenced by supplementation (trial effect: $F_{(2, 26)} = 4.99$, $P = 0.01$, $\eta_p^2 = 0.28$). Post hoc tests revealed that mean heart rate was higher in the CHO10 trial (169 ± 2 $b \cdot min^{-1}$) compared with the PLA trial (164 ± 3 $b \cdot min^{-1}$). The pattern of response in peak heart rate was affected by supplementation during the SMS protocol (trial × time effect: $F_{(14, 182)} = 1.77$, $P = 0.04$, $\eta_p^2 = 0.12$). Post hoc tests revealed that peak heart rate was higher in the CHO10 compared with the PLA trial at 15, 30, 45, 60 and 75 min ($P < 0.05$) (Figure 4.6).

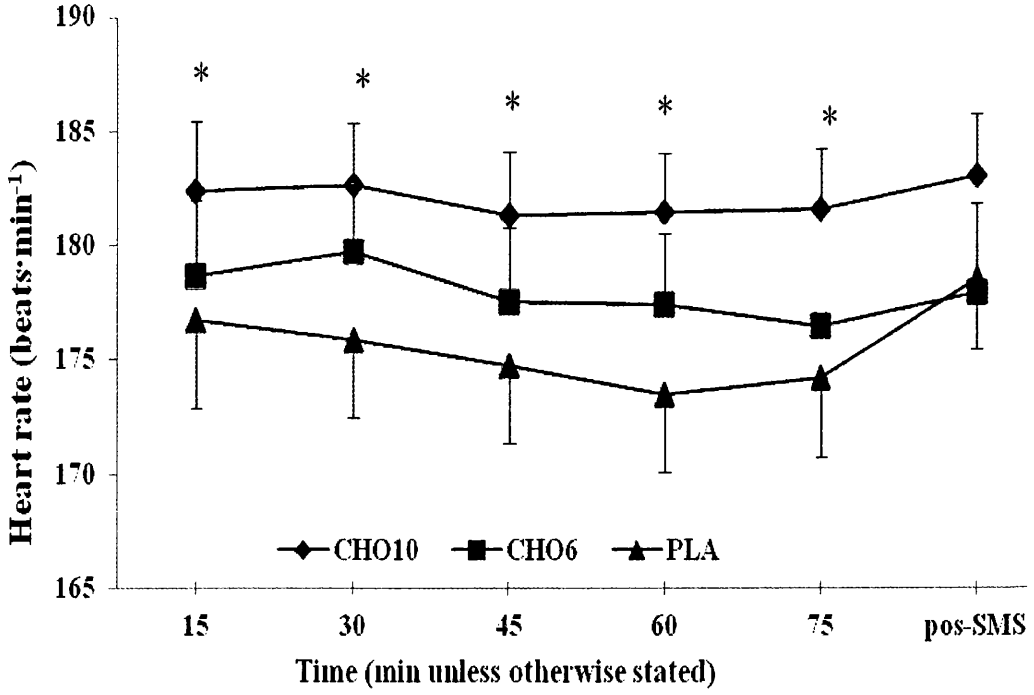


**Figure 4.6** Peak heart rate responses recorded during the SMS protocol. Values represent the mean ± *SEM*, $n$=14. * symbol indicates higher peak heart rate in the CHO10 trial compared with the PLA ($P$ <0.05).

The pattern of response for ratings of perceived exertion (RPE) were similar between trials during the SMS protocol (trial × time effect: $F_{(4.6, 60.4)} = 1.17$, $P = 0.33$, $\eta_p^2 = 0.08$). The ratings of perceived exertion were not affected by supplementation (trial effect: $F_{(2, 26)} = 0.60$, $P = 0.55$, $\eta_p^2 = 0.04$). However, the perceived exertion gradually increased due to the exercise performed throughout each half of the SMS protocol (time effect: $F_{(5, 65)} = 49.76$, $P < 0.001$, $\eta_p^2 = 0.79$). Post hoc tests revealed that the ratings of perceived exertions were higher at the end of the SMS protocol (post-SMS) compared with the rest of the time points across all trials ($P < 0.05$).

### 4.7 Sprint Speed

The pattern of response in sprint speed was similar between trials during the SMS protocol (trial × time effect: $F_{(4.4, 53.2)} = 0.40$, $P$ =0.94, $\eta_p^2 = 0.03$). Sprint speeds were not affected throughout the SMS protocol (time effect: $F_{(1.9, 23.0)} = 1.31$, $P$ =0.27, $\eta_p^2 = 0.10$). However, supplementation influenced sprint performance during the SMS

protocol (trial effect: $F_{(2, 24)} = 3.49$, $P < 0.05$, $\eta_p^2 = 0.22$). Post hoc tests revealed that sprint speed was 3.3% higher in the CHO10 trial compared with the PLA trial at 45 min.

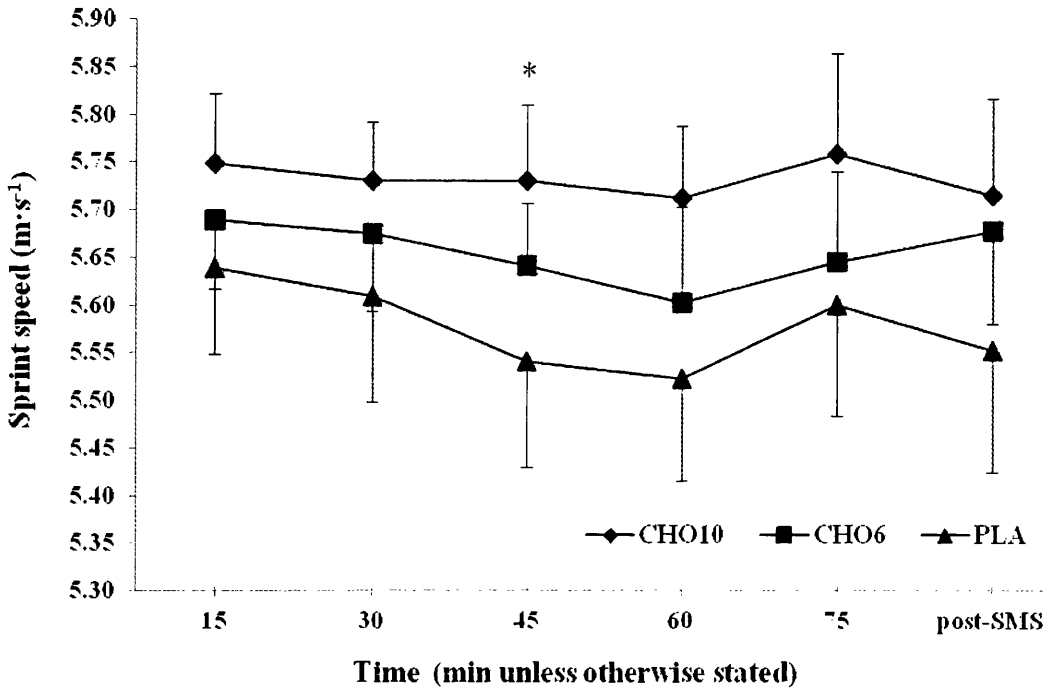


**Figure 4.7** Sprint speeds recorded during the SMS protocol. Values represent the mean ± *SEM*, *n*=14. * symbol indicates higher sprint speeds in the CHO10 trial compared with the PLA ($P < 0.05$).

# Chapter 5

## Discussion

## 5.0 Discussion

The main findings of the present study were: (1) the ingestion of carbohydrate and caffeine in the CHO10 treatment was effective in elevating blood glucose concentration at 15, 30, 45, 75 min and post-SMS time-points when compared with the PLA treatment. Carbohydrate supplementation in the CHO6 treatment was effective elevating blood glucose concentration at 45 min and post-SMS when compared with the PLA treatment. Additionally, supplementation with carbohydrate (CHO6) and co-ingestion of caffeine and carbohydrate (CHO10) did not attenuate the drop in blood glucose concentration observed across all trials after the half-time recovery period; (2) Sprint performance was enhanced at 45 min in the CHO10 treatment in comparison with the PLA treatment. This coincided with an increase in blood lactate concentration and peak heart rate in the CHO10 treatment and an increase in blood glucose concentration; (3) Plasma osmolality was higher post-SMS in the CHO10 and plasma sodium concentration was higher in the CHO10 and CHO6 trials when compared with the PLA treatment. However, plasma osmolality and sodium concentration values did not reach critical (dehydration) values; and (4) Body mass losses were similar between trials and the fluid ingestion rate limited body mass losses to <1% body mass across all trials.

### 5.1 Initial Nutritional and Physiological Status

The nutritional and physiological analysis revealed that participants started each main trial in a similar status. The data extracted from the participants' diet records revealed that the previous nutritional and energy intake was similar between trials. The participants ingested an average of 7070 ± 466 kJ, where 48 ± 2% were CHO, 35 ± 2% were fats and 17 ± 1% were proteins. This low dietary energy intake was probably caused by the participants underreporting their food intakes in the diet records. However, participants ingested a similar breakfast two hours before each main trial, made up of 500 ml of the treatment drink and foods providing 1470 kJ (62% carbohydrates, 25% fats, 13% proteins). The ingestion of a high-carbohydrate meal 2-3 hours before training and competition has been widely recommended to reduce the

effects of fatigue during exercise (FIFA, 2006; Williams and Serratosa, 2006). Therefore, the ingestion of a high-carbohydrate meal before each main trial followed the common recommendation for food and fluid intake before exercise, and increased the ecological validity of the study. In contrast, several studies have used glycogen depleting routines combined with low carbohydrate diets before exercise to enhance the effects of carbohydrate supplementation during exercise (Ali *et al.*, 2007, Ali and Williams, 2009; Nicholas *et al.*, 1999; Nicholas *et al.*, 1995; Phillips *et al.*, 2010). The findings of these studies cannot be applied to real settings, because the nutritional practices and glycogen depleting routines completed before the exercise protocol did not represent the current recommendations to enhance performance before games.

Initial urine osmolality suggested that participants arrived to laboratory in a similar hydrated state across all trials (PLA: 799 ± 48 $mosm \cdot l^{-1}$; CHO6: 722 ± 66 $mosm \cdot l^{-1}$; CHO10: 842 ± 53 $mosm \cdot l^{-1}$). The mean urine osmolalities indicated that participants arrived to the laboratory with mild dehydration, as mean osmolalities ranged between 700 $mosm \cdot l^{-1}$ (euhydration) and 900 $mosm \cdot l^{-1}$ (2% water deficit) (Sawka *et al.*, 2007; Shirreffs and Maughan, 1998). However, the first morning urine sample is typically more concentrated (higher osmolality) because individuals do not usually ingest any fluids or void their bladder overnight (Armstrong *et al.*, 2010). In order to standardise the participants' hydration status, they ingested 500 ml of the treatment beverage at least 2 hours before the main trials, following the recommendations in Shirreffs (2010); therefore, it is likely that participants were similarly euhydrated before all trials.

The initial plasma osmolality also indicated that participants arrived to the laboratory in a similar hydrated state (PLA: 292 ± 3 $mosm \cdot l^{-1}$; CHO6: 291 ± 2 $mosm \cdot l^{-1}$; CHO10: 292 ± 3 $mosm \cdot l^{-1}$). It has been suggested that plasma osmolalities between 280 and 300 $mosm \cdot l^{-1}$ indicate euhydration (Hamilton and Bickle, 2006; Weinberg and Minaker, 1995); therefore, the initial plasma osmolality values confirmed that participants arrived to the laboratory in a euhydrated state. Many studies have used plasma and urine osmolality to assess hydration status before exercise (Popowski *et al.*, 2001; Shirreffs and Maughan, 1998; Silva *et al.*, 2011) and the results suggest that most athletes start training and competition in a euhydrated state but a small minority do not. In the present study, it was likely that the ingestion of 500 ml of the treatment beverage 2 hours before

the SMS protocol was sufficient to ensure that the participants started all exercise trials in a similarly hydrated state (Shirreffs, 2010).

### 5.2 Blood Glucose Response

The ingestion of carbohydrates as sports drinks or gels has advocated the maintenance of homeostasis during exercise, preserving optimal levels of glycogen and glucose availability for muscle and brain metabolism. Blood glucose is an important fuel carried in the blood circulation and utilised by muscle, liver, kidney, red blood cells and lymphocytes, among others (Widmaier *et al.*, 2011). High concentrations of fasting blood glucose (>7 $mmol \cdot l^{-1}$) may result in the catabolism of muscle proteins and ketosis during acute hyperglycaemia (Jellinger, 2007). Similarly, low levels of blood glucose (<3.5 $mmol \cdot l^{-1}$) can negatively affect exercise performance, because it can cause sweating, inability to concentrate, confusion, tiredness, and if prolonged may lead to fainting, coma and sudden death (Frier, 2002). Therefore, the maintenance of blood glucose homeostasis during exercise seems not only important to preserved exercise performance, but also to avoid clinical problems.

Hypoglycaemia has been associated with the onset of fatigue during prolonged exercise (Fitts, 1994). Additionally, hypoglycaemia has been associated with the deterioration in soccer performance, which requires tactical thinking and visual identification of players and targets (Shephard and Leat, 1987). Generally, normal blood glucose levels are maintained during soccer games. However, several studies have reported low levels of blood glucose at half-time (Krustrup *et al.*, 2006) and at the end of the match (Ekblom, 1986). A study by Russell *et al.* (2011b) reported important reductions in blood glucose concentration at the beginning of the second half when compared with the initial values (17% and 19% reductions) during a soccer match and a soccer match simulation protocol. This was the first study to identify an exercise-induced rebound glycemic response during intermittent exercise while players ingested a carbohydrate-free electrolyte beverage. This decrease in blood glucose concentration may translate in a reduction of muscle glucose uptake and a decrease in exercise performance.

In the present study, the carbohydrate supplementations administered in the CHO10 and CHO6 conditions were effective elevating blood glucose concentrations when compared with PLA. Blood glucose concentrations were significantly higher in the CHO10 trial throughout the exercise protocol (15, 30, 45, 75 min and post-SMS), probably due to the additional dose of carbohydrates ingested within the CHO10 beverage and possibly due to the effects of caffeine ingestion on intestinal glucose absorption. Additionally, blood glucose concentrations were significantly higher in the CHO6 trial at 45 min and post-SMS when compared with the PLA trial. Similarly, several studies have observed that blood glucose concentration was elevated after the ingestion of a 6.4% carbohydrate-electrolyte beverage compared with a flavoured placebo during high-intensity intermittent exercise (Ali *et al.*, 2007; Backhouse *et al.*, 2007; Foskett *et al.*, 2008).

Interestingly, blood glucose concentrations were similarly challenged after the half-time recovery period. Blood glucose concentrations were 40%, 38%, and 16% lower in the CHO10, CHO6 and PLA trials respectively, at 60 min when compared with the 45 min time point. Interestingly, blood glucose concentrations were below 3.5 $mmol \cdot l^{-1}$ in four of the fifteen individuals after the ingestion of carbohydrate. Similar responses (rebound hypoglycaemia) have been observed at the onset of exercise after ingestion of carbohydrate (Sherman *et al.*, 1991; Kuipers *et al.*, 1999) and after the ingestion of a non-carbohydrate drink (Russell *et al.*, 2011b). This response was probably originated by the combined effect of an increased insulin concentration due to carbohydrate ingestion, an elevated muscle glucose uptake during the recovery period due to the effects of the exercise bout, and a reduction in the liver glucose output.

The use of a higher dose of carbohydrates plus caffeine in the CHO10 trial elevated blood glucose levels when compared with the PLA (15, 30, 45, 75 min, post-SMS) and the CHO6 trials (45, 75 min). However, the ingestion of carbohydrate in the CHO10 trial did not attenuate the blood glucose drop that has been observed during the first instances of the second half. Several studies have observed a decrease in exercise performance immediately after the half-time recovery period during soccer games (Bangsbo *et al.*, 1991; Mohr *et al.*, 2003; Bradley *et al.*, 2009; Weston *et al.*, 2011). This decrease in performance has been associated with the declines in muscle temperature caused by the 15-min passive recovery period (half-time). However, the

present study suggests that the decrease in performance during the first instances of the second half might be also related with low blood glucose concentrations.

### 5.3 Sprint Performance

High-intensity activities such as sprints, dribbles or jumps, account for 30% of the match-play time (Mohr *et al.*, 2003) and are commonly associated with crucial actions of the game (Reilly, 1997). The ingestion of carbohydrate beverages and caffeinated carbohydrate beverages has been associated with faster sprint performances during a soccer-specific exercise protocol (Ali *et al.*, 2007; Gant *et al.*, 2010). These studies associated the sprint performance improvements to the maintenance of blood glucose concentrations with the ingestion of carbohydrate (Ali *et al.*, 2007) and the diminished perception of fatigue associated with caffeine ingestion (Gant *et al.*, 2010). Conversely, several studies have observed that sprint performance was not affected by carbohydrate ingestion (Nicholas *et al.*, 1995; Morris *et al.*, 2003; Ali and Williams, 2009; Phillips *et al.*, 2010).

In the present study, mean sprint speed was faster in the CHO10 trial ($5.73 \pm 0.08$ m·s$^{-1}$) when compared with the CHO6 ($5.59 \pm 0.09$ m·s$^{-1}$) and PLA ($5.58 \pm 0.11$ m·s$^{-1}$), but this was not statistically significant. Additionally, sprint performance was enhanced at 45 min after supplementation with CHO10 in comparison with PLA ($P < 0.05$). This coincided with an increase in blood glucose concentration in the CHO10 trial, which could provide an increase availability of energy for muscle activation. Although the present study does not allowed the confirmation of the underlying factors enhancing sprint performance, it is suggested that the high concentrations of glucose ingested with the CHO10 beverage and the possible increase in intestinal absorption and carbohydrate oxidation due to caffeine intake could improve sprint performance in the present study (Yeo *et al.*, 2005). The analgesic effects of caffeine in the central nervous system could reduce the subjective perceptions of fatigue and allowed participants to perform faster sprints (Fredholm *et al.*, 1999; Motl *et al.*, 2006).

## 5.4 Hydration Indices

Exercise-induced dehydration is considered as one of the main factors causing fatigue in many sports and physical activities, especially in hot and humid conditions. Moderate losses in body water (1-2% BM) can increase the physiological and metabolic strain and compromise exercise and mental performance during exercise (Maughan and Leiper, 1994; Reilly, 1997; Sawka *et al.*, 2007). Several studies have indicated that dehydration decreases prolonged aerobic exercise performance (Armstrong *et al.*, 1985; Casa *et al.*, 2005; Cheuvront *et al.*, 2005; Walsh *et al.*, 1994). However, not many studies have investigated the effects of dehydration on soccer-specific exercise performance. McGregor *et al.* (1999) showed that soccer skills performance (dribbling test) deteriorated when fluid ingestion was denied, however, skills performance was maintained throughout the exercise protocol when fluids were ingested. The decrease in skills performance observed in the dehydrated group was associated with an increase in cardiovascular strain (blood volume and stroke volume reductions, increase heart rate, increase blood viscosity) and electrolyte imbalance (increased sodium concentration and plasma osmolality). Similarly, Edwards *et al.* (2007) suggested that the decrease in intermittent exercise performance in dehydrated individuals was associated with an increase in plasma osmolality and core temperature, probably originated by significant body water losses (2.4% of initial body mass). In order to avoid water deficits in excess of 2% of body mass and the negative physiological and physical effects of dehydration, individuals engaged in prolonged exercise (>1 hour) should ingest an adequate volume of fluids before and during exercise (Sawka *et al.*, 2007).

In the present study, sweat losses were similar between trials (PLA: 2.0 ± 0.1 l; CHO6: 1.9 ± 0.1 l; CHO10: 2.0 ± 0.1 l). Fluid ingestion was also similar between trials (PLA, CHO6, CHO10 = 1.9 l). Therefore, the volume of fluids ingested was similar to the amount of body water lost during the exercise protocol. It should be noted that this fluid ingestion rate was in excess to the participants' usual ingestion during competition and that was at the limit of tolerance, as some of the participants experienced a certain degree of bloating and abdominal discomfort during the exercise protocol.

The main purpose of ingesting carbohydrate-electrolytes beverages (sport drinks) before or during exercise is to replace fluid losses and provide a readily available fuel for muscle and brain metabolism. The ingestion of sport drinks has been widely recommended to postpone fatigue, maintain blood glucose and muscle glycogen concentrations and preserved an adequate level of hydration (Casa *et al.*, 2000; Sawka *et al.*, 2007). However, increasing the carbohydrate concentration (>8%) and osmolality of the ingested beverage can delay gastric emptying and fluid absorption at rest and during exercise (Jeukendrup, 2008). Several studies have reported that an increase in osmolality due to high carbohydrate concentrations produced a net secretion of water into the intestinal lumen, reducing the body water pool and increasing the effects of dehydration (Gisolfi *et al.*, 1992; Leiper and Maughan, 1986). Evans *et al.* (2009) showed that the ingestion of a hypertonic 10% glucose solution (565 $mosm \cdot l^{-1}$) significantly reduced plasma volume and increased plasma osmolality compared with the ingestion of a 2% glucose solution (111 $mosm \cdot l^{-1}$) at rest. They suggested that the decrease in plasma volume after ingestion of a 10% glucose solution was caused by a temporary secretion of water from the blood into the intestinal lumen. This response can increase peripheral resistance and cardiovascular strain, which might negatively affect exercise performance, especially in hot and humid conditions. In contrast, several studies have reported similar physiological responses (plasma volume changes, plasma osmolality, plasma sodium concentration) and fluid delivery at rest (Davids *et al.*, 1990) and during an intermittent cycling protocol (Murray *et al.*, 1989) when ingesting sports drinks that varied in their carbohydrate concentrations (6%, 8% and 10%).

In the present study, the ingestion of a 10% carbohydrate-electrolyte beverage plus a carbohydrate gel (CHO10) resulted in a 2% increase in plasma osmolality at the end of the SMS protocol (pre-SMS: 292 ± 3 $mosm \cdot l^{-1}$; post-SMS: 298 ± 3 $mosm \cdot l^{-1}$, $P< 0.05$). Additionally, the ingestion of a 6% carbohydrate-electrolyte beverage plus a carbohydrate gel tended to increase plasma osmolality at the end of the SMS protocol (pre-SMS: 290 ± 2 $mosm \cdot l^{-1}$; post-SMS: 296 ± 3 $mosm \cdot l^{-1}$, $P= 0.06$). This response was accompanied by a significant elevation of plasma sodium concentration in the CHO10 and CHO6 trials when compared with the PLA trial at different time points. Additionally, plasma volume followed a similar pattern across all trials. Plasma volume decreased at the onset (15 min) of exercise and remained unchanged in the CHO10 and

CHO6 trials and increased in the PLA trial towards the end of the SMS protocol (75 min and post-SMS).

These findings suggest the ingestion of 6% and 10% carbohydrate-electrolyte solutions (CHO6 and CHO10) resulted in higher plasma osmolalities and higher sodium concentration when compared with the ingestion of a non-carbohydrate beverage (PLA). These findings are in agreement with the study of Evans *et al.* (2009), where the ingestion of a 10% glucose solution at rest resulted in a decrease of plasma volume and an increase of plasma osmolality. However, this study investigated the blood and plasma responses after ingestion of beverages differing in carbohydrate concentration and osmolality at rest. In the present study, blood and plasma responses were investigated during and after exercise therefore the plasma responses were probably caused by the combined effects of exercise, carbohydrate and fluid ingestion.

Regardless of plasma sodium concentration and plasma osmolality being higher in the CHO10 and CHO6 compared with the PLA treatment, the mean values recorded were lower than the critical values considered as hypertonic dehydration (sodium concentration: >145 $mmol \cdot l^{-1}$; plasma osmolality: >300 $mosm \cdot l^{-1}$) (Hamilton and Bickle, 2006; Weinberg and Minaker, 1995). Therefore, it seems that the ingestion of highly concentrated carbohydrate-electrolyte beverages (CHO10) did not jeopardize hydration status during the SMS protocol. The use of multiple types of transportable carbohydrates (e.g. glucose, fructose) in carbohydrate-electrolytes beverages has been associated with enhances intestinal absorption of fuels and fluids (Jentjens *et al.*, 2005b; 2006; Lambert *et al.*, 2008). Therefore, the composition (maltodextrin and fructose) and low solution osmolality (CHO10: $292 \pm 3$ $mosm \cdot l^{-1}$; CHO6: $112 \pm 15$ $mosm \cdot l^{-1}$) of the 10% and 6% carbohydrate beverage probably facilitated fluid and fuel delivery during the exercise protocol. In addition, it should be considered that the exercise trials were performed under temperate conditions (18.7 C° and 61% humidity), where exercise-induced water losses are less prominent than the ones observed during exercise in hot and humid conditions (Kurdak *et al.*, 2010; Mustafa and Mahmoud, 1979). Therefore, individuals should be cautious when ingesting highly concentrated carbohydrate beverages and exercising in hot and humid conditions.

# Chapter 6

## Conclusion and Limitations

## 6.0 Conclusion

Carbohydrate supplementation (CHO10 and CHO6) was effective in elevating blood glucose concentrations during an intermittent high-intensity protocol that replicated soccer match-play. The additional dose of carbohydrate and the addition of caffeine to the beverage in the CHO10 trial were not successful in attenuate the drop in blood glucose observed after the half-time recovery period. Therefore, further studies should aim to investigate supplementation or exercise strategies to attenuate or eliminate acute drops in blood glucose concentrations. If players' blood glucose concentrations reach hypoglycaemic ($<3.5$ $mmol \cdot l^{-1}$) values during the first instances of the second half, exercise and skill performance can be negatively affected, increasing the chances of conceding goals.

Carbohydrate supplementation, especially the ingestion of a highly concentrate carbohydrate beverage with caffeine (CHO10) increased plasma osmolality and sodium concentration throughout the SMS exercise protocol. These responses have been associated with a decrease in the body water pool, which in turn can result in hyperthermia and an increase in the cardiovascular strain, ultimately affecting exercise performance. However, the mean values recorded for plasma osmolality and sodium concentration did not indicate that players suffered an important degree of dehydration during the exercise protocol. It seems that 10% carbohydrate beverages can be used during soccer-specific activities in temperate conditions; however, players should be cautious when ingesting a 10% carbohydrate beverage in hot and humid conditions because it can accelerate the dehydration process.

The ingestion of a 10% carbohydrate with caffeine resulted in faster sprint performance. The small improvement with the ingestion of a 10% carbohydrate caffeinated drink may represent important performance improvements during match-play, where faster running speed may imply reaching the ball from a team mate pass or blocking a pass or shoot of an opponent player.

## 6.1 Limitations and Future Recommendations

Although the soccer-specific protocol is a close representation of the requirements of a soccer game (Russell *et al.*, 2011a), the variability of match-play cannot be replicated in an exercise protocol; therefore, it is not possible to replicate the stresses of competition and the self-pacing strategies of players during a game. Additionally, the completion of the SMS protocol in an indoor facility with an artificial surface meant that the conditions were replicated in all trials; however, most soccer matches are played on grass pitches in variable environmental conditions. Future studies could aim to evaluate the influence of carbohydrate concentrations on hydration status in different environmental conditions.

The energy intakes reported in the study were low for active individuals that are involved in physical activity on a daily basis. The possible cause of this low energy intake could be the participants underreporting their foods and drinks intakes, as this will affect the total energy intake and the contributions of the different types of macronutrients to it.

The assessment of the rate of gastric emptying and intestinal fluid absorption were not measured in the present study. These assessments could provide important information about the physiological responses after the ingestion of a higher dose of carbohydrate mixed with caffeine and its effects on hydration during exercise.

The rate of fluid ingestion utilised in the study seemed to limit body mass losses to less than 1% of body mass. However, this fluid ingestion was in excess to the participants' usual ingestion during training and competition. Future studies should be aimed to identify the relation between individual differences (sweat rates, acclimatisation, and aerobic fitness) with the individual fluid intake during exercise in different environmental conditions.

Based on the study results, the ingestion of fluids with carbohydrates and caffeine is recommended to increase sprint performance and elevate blood glucose concentration. However, the ingestion of highly concentrated carbohydrate beverages seemed to increase the concentration of the extracellular body fluids, response that can be associated with a decrease of the body water pool. The hydration indices recorded in the study did not reach critical values; however, soccer players should be cautious when ingesting highly concentrated carbohydrate drinks and exercising in hot and humid conditions. Further studies investigating supplementation with higher dose of carbohydrates in hot and humid conditions will offer attractive research opportunities.

One of the aims of this study was to investigate the metabolic and hydration effects of ingesting additional caffeinated carbohydrate beverages in a group of recreational players during soccer-specific activities. However, the findings may not transfer directly to professional soccer players as the group of participants in the present study were all recreational soccer players. It is possible that the physiological characteristics of professional soccer players responded differently to the supplementation strategies; therefore, the investigation of the physiological and metabolic effects with co-ingestion of carbohydrate and caffeine in a group of elite soccer players will provide an attractive research opportunity.

# Chapter 7

## Appendices

**APPENDIX A:** Ethics Proposal

**Swansea University**

**SPORTS SCIENCE, SCHOOL OF HUMAN SCIENCE**

**DEPARTMENTAL ETHICS ADVISORY COMMITTEE**

## APPLICATION FOR ETHICAL COMMITTEE APPROVAL OF A RESEARCH PROJECT

In accordance with Departmental Safety Policy, all research undertaken in the department must be approved by the Departmental Ethics Advisory Committee **prior to** data collection. **Applications for approval should be typewritten on this form using the template available in the Public Folders.** The researcher(s) should complete the form in consultation with the project supervisor. Where appropriate, the application must include the following appendices:

(A) subject information sheet;

(B) subject consent form;

(C) subject health questionnaire.

**After completing sections 1-12 of the form, 1 copy of the form should be handed-in to the Department Administrator who will then submit copies of the application for consideration by the Departmental Ethics Advisory Committee. The applicant(s) will be informed of the decision of the Committee in due course.**

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### 1. DRAFT TITLE OF PROJECT

The effects of ingesting a larger dose of carbohydrate on metabolic responses during a soccer-specific exercise protocol.

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### 2. NAMES AND STATUS OF RESEARCH TEAM

Dr. Mike Kingsley – Supervisor

Carlos Penas Ruiz – Postgraduate student

Chris Terry – Postgraduate student

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### 3. RATIONALE

It has been widely identified that a decrease in muscle glycogen and blood glucose availability during soccer games can lead to fatigue (Saltin, 1973; Mohr *et al.*, 2005; Krustrup *et al.*, 2006). Fatigue, especially towards the end of the game can lead to impairments in exercise (Mohr *et al.*, 2003; Rampinini *et al.*, 2007) and soccer skills performance (McGregor *et al.*, 1999; Rampinini *et al.*, 2008; Ali *et al.*, 2009). Interestingly, most of the goals are scored in the last 15 minutes of the game, which may be related with the earlier mentioned decrease in exercise and skills performance (Reilly, 1996).

The provision of carbohydrate (CHO) appears to induce metabolic and perceptual benefits during actual soccer game or soccer match simulations (Coyle *et al.*, 1986; Nicholas *et al.*, 1995; Ostojic and Mazic, 2002; Ali *et al.*, 2009). Sports drinks are usually ingested to prevent dehydration, preserve muscle glycogen and blood glucose levels, and replace electrolytes losses during exercise. However, the optimal characteristics of sports drinks (type, amount and concentration of CHO) are still being debated.

Exogenous CHO oxidation seems to be limited to rates of 1.0 to 1.1g • $min^{-1}$ (Wagenmakers *et al.*, 1993; Bosch *et al.*, 1994; Jeukendrup *et al.*, 1997). However, it has been shown that large doses (2.4 g • $min^{-1}$) of a blend of different types of CHO can enhance CHO oxidation rates (~1.7 g • $min^{-1}$) if compared with the administration of a single source of carbohydrate (Jentjens, Achten and Jeukendrup, 2004; Jentjens and Jeukendrup, 2005a).

It has been suggested that an increase in the concentration of CHO in sport drinks may lead to a decrease in fluid availability therefore causing dehydration (Maughan and Leiper, 1999). The increase in beverage osmolality originates a water displacement from tissues to the intestinal lumen causing a loss in the body water pool (Gisolfi *et al.*, 1990). Davids *et al.* (1990) suggested that the ingestion of CHO beverages with concentrations ranging between 3% and 10% were not likely to reduce fluid availability if compared with water ingestion. However, a recent study by Jeukendrup *et al.* (2009) showed that fluid availability was compromised with CHO solutions concentration above 6%.

Typically, caffeine has been ingested by athletes because its reported ergogenic effects (Ganio *et al.*, 2009). The ingestion of carbohydrate with small doses of caffeine has been shown to increase exogenous CHO oxidation (Yeo *et al.*, 2005), maintain optimal blood glucose levels (Cureton *et al.*, 2007), and increase intestinal CHO absorption (Van Nieuwenhoven *et al.*, 2000) during exercise. However, there has been cases where the ingestion of CHO with caffeine (5.3 mg • $kg^{-1}$) did not influence exogenous CHO oxidation or glucose responses during exercise (Hulston and Jeukendrup, 2008).

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## 4. REFERENCES


Ali, A., and Williams, C. (2009). Carbohydrate ingestion and soccer skill performance during prolonged intermittent exercise. Journal of Sport Sciences, 27(14), 1499-1508.

Bosch, A.N., Dennis, S.C., and Noakes, T.D. (1994). Influence of carbohydrate ingestion on fuel substrate turnover and oxidation during prolonged exercise. Journal of Applied Physiology, 79, 2364-72.

Coyle, E.F., Coggan, A.R., Hemmert, M.K, and Ivy, J.L. (1986). Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. Journal of Applied Physiology, 61(1), 165-72.

Cureton, K.J., Warren, G.L., Millard-Stafford, M.L., Wingo, J.E., Trilk, J., and Buyckx, M. (2007). Caffeinated Sports Drink: Ergogenic Effects and Possible Mechanisms, International Journal of Sport Nutrition and Exercise Metabolism, 17, 35-55.

Davis, J.M., Burgess, W.A., Slentz, C.A., and Bartoli, W.P. (1990). Fluid availability and sport drinks differing in carbohydrate type and concentration. American Journal of Clinical Nutrition, 51, 1054-57.

Ganio, M.S., Klau, J.F., Casa, D.J., Armstrong, L.E., and Maresh, C.M. (2009). Effect of caffeine on sport-specific endurance performance: a systematic review. Journal of Strength and Conditioning Research, 23(1), 315–324.

Gisolfi, C.V., Summers, R.W., Schedl, H.P., Bleiler, T.L., and Oppliger, R.A. (1990). Human intestinal water absorption: direct vs. Indirect measurements. American Journal of Physiology, 258, 216-222.

Hulston, C.J. and Jeukendrup, A.E. (2008). Substrate metabolism and exercise performance with caffeine and carbohydrate intake. Medicine and Science in Sport and Exercise, 40(12), 2096-2104.

Jentjens, R.L., Achten, J., and Jeukendrup. A.E. (2004). High oxidation rates from combined carbohydrates ingested during exercise. Medicine and Science in Sport and Exercise, 36(9), 1151-58.

Jentjens, R.L., and Jeukendrup, A.E. (2005a). High rates of exogenous carbohydrate oxidation from a mixture of glucose and fructose ingested during prolonged cycling exercise. The British Journal of Nutrition, 93(4), 485-92.

Jeukendrup, A.E., Mensink, M., and Saris, W.H., and Wagenmakers, A.J. (1997). Exogenous glucose oxidation during exercise in endurance trained and untrained subjects. Journal of Applied Physiology, 82(3), 835-40.

Jeukendrup, A.E., Currell, K., Clarke, J., Cole, J., and Blannin, A.K. (2009). Effect of beverage glucose and sodium content on fluid delivery. Nutrition and Metabolism, 6, 9.

Krustrup, P., Mohr, M., Steensberg, A., Bencke, J., Kjær, M., and Bangsbo, J. (2006). Muscle and blood metabolites during a soccer game: Implications for sprint performance. Medicine and Science in Sport and Exercise, 38(6), 1165-1174.

Maughan, R.J., and Leiper, J.B. (1999). Limitations to fluid replacement during exercise. Canadian Journal of Applied Physiology, 24, 173-187.

McGregor, S.J., Nicholas, C.W., Lakomy, H.K., and Williams, C. (1999). The influence of intermittent high-intensity shuttle running and fluid ingestion on the performance of a soccer skill. Journal of Sports Sciences, 17, 895-903.

Mohr, M., Krustup, P., and Bangsbo, J. (2003). Match performance of high-standard soccer players with special reference to development of fatigue. Journal of Sports Sciences, 21(7), 519-28.

Mohr, M., Krustrup, P., and Bangsbo, J. (2005). Fatigue in soccer: a brief review. Journal of Sports Sciences, 23(6), 593-599.

Nicholas, C.W., Williams, C., Lakomy, H.K., Phillips, G., and Nowitz, A. (1995). Influence of ingesting a carbohydrate–electrolyte solution on endurance capacity during intermittent, high-intensity shuttle running. Journal of Sports Sciences, 13, 283-290.

Ostojic, S., and Mazic, S. (2002). Effects of a carbohydrate-electrolyte drink on specific soccer test and performance. Journal of Sports Science and Medicine, 1, 47-53.

Rampinini, E., Coutts, A.J., Castagna, C., Sassi, R., and Impellizzeri, F.M. (2007). Variation in top level soccer match performance. International Journal of Sports Medicine, 28, 1018-24.

Rampinini, E., Impellizzeri, F. M., Castagna, C, Azzalin, A., Bravo, D. F., and Wislkff, U. (2008). Effect of match-related fatigue on short-passing ability in young soccer players. Medicine and Science of Sports and Exercise, 40(5), 934-42.

Reilly, T., (1996). Motion analysis and physiological demands. London: E & FN Spon.

Saltin, B. (1973). Metabolic fundamentals in exercise. Medicine and Science in Sports, 5, 137-46.

Van Nieuwenhoven, M.A., Brummer, R.M., and Brouns, F. (2000). Gastrointestinal function during exercise: comparison of water, sports drink, and sports drink with caffeine. Journal of Applied Physiology, 89, 1079-1085.

Wagenmakers, A.J.M., Brouns, F., Saris, W.H., and Halliday, D. (1993). Oxidation rates of orally ingested carbohydrates during prolonged exercise in man. Journal of Applied Physiology, 75, 2774-80.

Yeo, S.E., Jentjens, R.L., Wallis, G.A. and Jeukendrup, A.E. (2005). Caffeine increases exogenous carbohydrate oxidation during exercise. Journal of Applied Physiology, 99, 844-50.

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## 5. AIMS and OBJECTIVES

The aim of the study is to assess the influence of different sport drinks and gels (with an increased dose of CHO + caffeine) on the metabolic responses and soccer skills performance during a soccer-specific exercise protocol.

***The objectives of the study are:***

- To evaluate the metabolic responses and soccer skills performance with the ingestion of a larger dose (above standard 6% CHO-electrolyte beverage) of carbohydrate during a soccer-specific exercise protocol.
- To evaluate the metabolic responses and soccer skills performance with the ingestion of an additional dose of carbohydrate (CHO gel) supplied during the half time period of a soccer-specific exercise protocol.
- To evaluate the metabolic responses and soccer skills performance with the co-ingestion of a larger dose of CHO + caffeine.

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## 6. METHODOLOGY

### 6.1 Study Design

A minimum of 15 participants will complete the described procedures of the study. The initial laboratory session will be used to estimate maximum oxygen uptake via the multistage fitness test (MSFT; Ramsbottom, Brewer & Williams, 1988), and the two remaining sessions will familiarise participants with the exercise regime and skill tests incorporated within the SMS protocol undertaken during the main experimental trials.

Following approval by the University ethics committee and informed consent being attained (parental consent where necessary; <18 years), players aged between 14 and 35 years old, and all with two or more years playing experience, will be recruited (diabetics or smokers will not participate). Four main trials (separated by no more than 14 days) will be completed in a randomised, double-blind and cross-over fashion. Supplementation will differ between trials, as follows: (trial A) 6% CHO-electrolyte solution with a placebo gel; (trial B) 6% CHO-electrolyte solution with CHO gel sachets; (trial C) 10% CHO-electrolyte and caffeine solution with CHO gel sachets, and (trial D) a control treatment ingesting only flavoured water. All players will be advised to refrain from strenuous physical activity and caffeine consumption during the three days before all testing sessions. Additionally, participants will be required to record all food consumption the previous day before each main trial. Food records will subsequently be analysed using commercially available software (CompEat version 5.8.0; Nutrition Systems, UK). At the completion of the study, all participants will be asked whether they had complied with all instructions.

### 6.2 Experimental Procedures

*Preliminary Testing*

Three preliminary testing sessions will be completed, and on each occasion arrival at the testing site will require participants to empty their bowels and void their bladder. In the first preliminary session, anthropometric measurements of body mass (model 770; Seca Ltd, Birmingham, UK) and stature (Portable Stadiometer; Holtain Ltd, Wales, UK) will be determined before commencing a controlled warm up that consists of 5 min of light aerobic activity and 10 min of dynamic stretching and sprints (that progress to near maximal speeds). Maximal oxygen uptake will then be estimated using the protocol outlined by Ramsbottom *et al.* (1988) in order to pair participants according to the intensity of the

exercise protocol to be used in the main trials (i.e., within 0.5 levels on multistage fitness test). The two remaining sessions will serve to familiarise participants with the procedures of the main trials; consequently, players will be familiarised with the exercise regime and skilled components of the SMS.

*Main Experimental Trials*

Participants will be required to attend the laboratory after an overnight fast at approximately 08:00 hours (i.e., 2.5 hours before commencing exercise). Upon arrival players will be prompted to provide a mid-flow urine sample and urine osmolality will subsequently be measured by freezing point depression (Gonotec Cryoscopic Osmometer Osmomat 030; YSI Limited, UK). A resting blood sample will then be taken before players consume a standardised 1470 kJ meal (Energy content: 62% carbohydrates, 25% fats, 13% proteins) and 500 ml of the supplement beverage at 08:30 hours. Body mass (model 770; Seca Ltd, Birmingham, UK) and stature (Portable Stadiometer; Holtain Ltd, Wales, UK) will then be measured. Players will remain in a rested state for approximately 100 min. A pre-exercise blood sample will be taken before players commence their final pre-exercise preparations by performing a standardised warm-up (consisting of running, dynamic stretching and ball skills) that precedes the starting the SMS. Supplement beverages will be consumed throughout all trials to supply 21 ml·kg $^{-1}$ BM (~ 14 ml· kg $^{-1}$ h $^{-1}$ BM); where 5.25 ml· kg $^{-1}$ BM will be consumed 10 min prior to commencing each half of the SMS and 2.63 ml· kg $^{-1}$ BM after 15, 30, 60 and 75 min of exercise. In addition, 2 x 38 g gel sachets will be consumed along with fluid 10 min prior to commencing each half of the SMS. A schematic of the trials is presented in Figure 1.

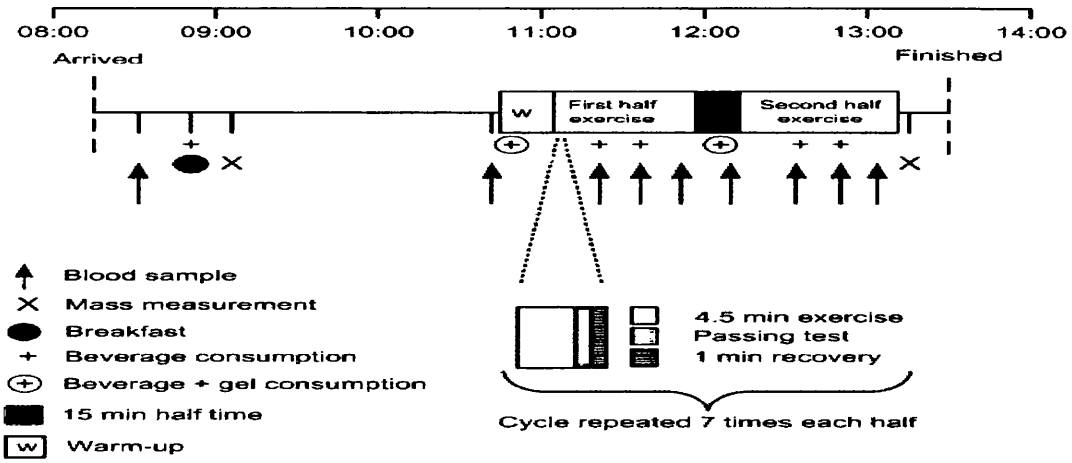


**Figure 1:** Schematic representation of the trial procedures.

*Soccer Match Simulation (SMS)*

The SMS requires participants to perform soccer skills throughout two ~47 min halves of soccer-specific activity that are separated by a 15-min passive recovery period (half-time). The exercise protocol is similar to that devised by Nicholas *et al.* (2000) but has subsequently adapted to include additional components that further replicate the movement demands of soccer match-play (Kingsley, Wadsworth, Kilduff, McEneny & Benton, 2005). Movements will be dictated by audio signals from CDs and each participant will alternate between sprinting and dribbling during each cycle.

More specifically, exercise is made up of 4.5-min blocks that consists of 3 repeated cycles of three 20-m walks, an alternating 15-m timed sprint (Brewer timing gates) or an 20-m dribble, a 4-s passive recovery period, five 20-m jogs at a speed corresponding to 40% $\dot{V}O2\,max$, one 20-m backwards jog at 40% $\dot{V}O2\,max$ and two 20-m strides at 85% $\dot{V}O2\,max$. A 2 min period incorporating the performance of soccer passing (1 min) and recovery (1 min) will follow all blocks of exercise. Seven blocks of intermittent activity and skills will be completed during each half of exercise. The participants cover a total distance of 10.1 km and will complete 56 passes and 21 dribbles during the protocol.

Figure 2 shows the schematic of the passing skill test. Balls (Total 90 Aerow: size 5; Nike Inc, USA) will be released at a constant velocity of 2.3 $m \cdot s^{-1}$ towards a 1.5 x 1.5-m square (action zone), where participants will be instructed to kick the ball. The participants kick towards one of two randomly determined targets (identified by a custom lighting system); consequently, the players are required to carry out visual searching and decision making during each attempt (similar to a soccer match when looking for space or other players). Motion sensors on the ball release mechanism ensure standardization and repeatability of each attempt; with a delay of 0.64 s between target identification and the ball reaching the centre of the action zone.

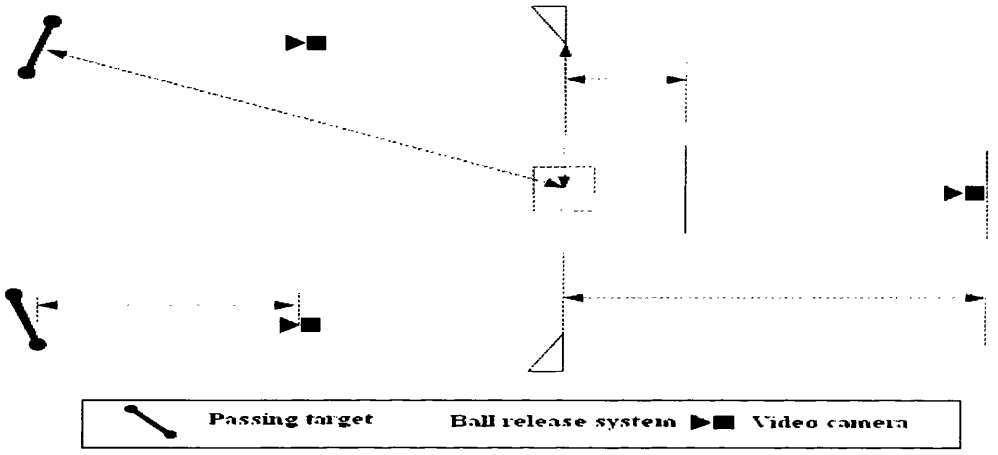


**Figure 2:** Schematic for the layout of the passing test.

Participants will commence the passing skill tests from a standing start before jogging into the action zone when the ball is released. The 2.0 x 1.0-m passing targets will be placed at distances of 7.9 m away from the centre of the action zone. The participants will be instructed to aim the ball at the centre of the target that is illuminated. The bouts of passing consist of four attempts, where the ball is alternately delivered from the right and left side of the action zone. To enhance ecological validity, no prior touches are allowed to control the ball (Olsen, 1988) and participants choose to kick the ball with the foot that they feel is most suitable to successfully complete the task.

The dribbling task is similar to that employed by McGregor *et al.* (1999) with start and finish lines placed 20-m apart. Cones 2 through 7 are placed 3-m away from the preceding cone, and cones 1 and 7 are 1-m away from each end of the course. Participants will be instructed to dribble the ball as fast and as accurately as possible.
Video footage will be captured using 50 Hz video cameras (DCR-HC96E; Sony Ltd, UK) that will be placed 0.5 m above the ground in the positions shown in Figure 1 (passing). All cameras will be synchronised using an audio signal and maximal target to camera distances will be used in order to minimise parallax errors within the field of view. Passing performances will be represented in terms of the success; a variable that has been shown to be reliable and demonstrate construct validity (unpublished observations). Success in passing represents those skills executed within the confines of the action zone and where the ball impacts upon the correct target box.

*Physiological testing*

Capillary blood samples will be taken at the following time points: rest, pre-exercise and following the start of exercise; 15, 30, 45, half-time, 60, 75 and 90 min. The GEM Premier 3000 analyser (GEM Premier 3000 blood gas analyser, Instrumentation Laboratory, UK) will be used to immediately analyse 170 μL of whole blood at each time point for pH, $Na^+$, $K^+$, $Ca^{2+}$ haematocrit, glucose and lactate concentrations.

Exercising HR will be recorded every 5 s using short range telemetry (Polar S610 HR monitor, Polar, Finland). Additional variables of plasma osmolality (Gonotec Cryoscopic Osmometer Osmomat 030; YSI Limited, UK), haemoglobin (HemoCue AB, Sweden), and urine corrected mass changes will be determined and the rate of perceived exertion (Borg, 1973) will be recorded throughout each trial.

*Supplementation*

During the main experimental trials participants will initially consume (with the standardised pre-exercise meal) 500 ml of beverage. Supplement beverages will be consumed throughout all trials to supply 21 ml·kg$^{-1}$ BM (~ 14 ml· kg$^{-1}$ ·hr$^{-1}$ BM), with 5.25 ml· kg$^{-1}$ BM will be consumed 10 min prior to commencing each half of the SMS and 2.63 ml· kg$^{-1}$ BM after 15, 30, 60 and 75 min of exercise. In addition, 2 x 38 g gel sachets will be consumed along with fluid 10 min prior to commencing each half of the SMS. The beverage and gel will differ between the three trials, as follows: (trial A) 6% CHO-electrolyte solution with placebo gel; (trial B) 6% CHO-electrolyte solution with CHO gel; and (trial C) 10% CHO-electrolyte and caffeine solution with CHO gel sachets. The total carbohydrate and caffeine ingestion during each of the trials is described in table 1.

**Table 1:** Total Carbohydrate and caffeine contain of the three experimental conditions during breakfast and exercise.

| Trial | Supplement | Breakfast | Exercise | Total (Inc. Breakfast) |
|---|---|---|---|---|
| **A** | Fluid (ml • kg$^{-1}$) | ~7.8 | 21 | ~28.8 |
| | Carbohydrate (g • kg$^{-1}$) | ~0.47 | 1.26 | 1.73 |
| | Caffeine (mg • kg$^{-1}$) | 0 | 0 | 0 |
| **B** | Fluid (ml • kg$^{-1}$) | ~7.8 | 21 | ~28.8 |
| | Carbohydrate (g • kg$^{-1}$) | ~0.47 | 2.70 | 3.17 |
| | Caffeine (mg • kg$^{-1}$) | 0 | 0 | 0 |
| **C** | Fluid (ml • kg$^{-1}$) | ~7.8 | 21 | ~28.8 |
| | Carbohydrate (g • kg$^{-1}$) | ~0.78 | 3.54 | 4.32 |
| | Caffeine (mg • kg$^{-1}$) | ~2.34 | 6.3 | 8.64 |
| **D** | Fluid (ml • kg$^{-1}$) | ~7.8 | 21 | ~28.8 |
| | Carbohydrate (g • kg$^{-1}$) | 0 | 0 | 0 |
| | Caffeine (mg • kg$^{-1}$) | 0 | 0 | 0 |

### 6.3 Data Analysis Techniques

Version 16 of the SPSS data collection programme will be used to analyse all data. Differences between trials will be assessed using two-way repeated measure ANOVA with intra-class correlation coefficients.

### 6.4 Storage and Disposal of Data and Samples

All data will be recorded and kept on a Microsoft Excel document in strict accordance with the Data Protection Act. All data will remain anonymous and will only be used for the purpose of the study. The data will be kept on a password locked user area on the Swansea University database, again under strict confidentiality. The data will only be accessible from the researchers (Carlos Penas Ruiz) and the supervisor of the study (Dr. Mike Kingsley).

Furthermore, all data will be deleted at the end of the study.

### 6.5 Dietary supplementation

(a) Several types of carbohydrate in different forms will be ingested during the main trials. The beverage in trial A, B and C contains a blend of maltodextrin, glucose and fructose. In addition, the

beverage in trial C contains caffeine. The active gel ingested during trial B and C contains a blend of maltodextrin and glucose.

(b) All the supplements will be provided by: High Five, Unit 4, Ash Court, Forrest Business Park, Bardon, Leicester, Leicestershire, LE67 1UD.

(c) During the main experimental trials participants will initially consume (with the standardised pre-exercise meal) 500 ml of beverage. Supplement beverages will be consumed throughout all trials to supply 21 ml·kg$^{-1}$ BM (~ 14 ml· kg$^{-1}$ ·hr$^{-1}$ BM), with 5.25 ml· kg$^{-1}$ BM will be consumed 10 min prior to commencing each half of the SMS and 2.63 ml· kg$^{-1}$ BM after 15, 30, 60 and 75 min of exercise. In addition, 2 x 38 g gel sachets will be consumed along with fluid 10 min prior to commencing each half of the SMS.

(d) All beverages and gels will be ingested orally.

(e) The ingestion of a carbohydrate gel during the recovery period (15 min half time) is expected to maintain blood glucose concentration during the second half of the exercise protocol and therefore decrease the use of endogenous carbohydrates. In addition, the typical exercise and soccer skills performance decrease suffered during the second half of the protocol is expected to be attenuated with the ingestion of additional carbohydrates at half time (CHO gel).

It is also expected that a large dose of carbohydrate (10%) ingestion will improve exercise and soccer skills performance without jeopardise hydration levels.

(f) The ingestion of carbohydrate has not been associated with important health problems however; a very small number of research studies have shown that ingestion of carbohydrate during endurance running may cause gastrointestinal discomfort (van Nieuwenhoven *et al.*, 2005; Pfeiffer *et al.*, 2009).

The ingestion of large doses of caffeine may produce insomnia, nervousness, irritability, anxiety and wakefulness during resting conditions (Jacobson and Kullin, 1989). However, the caffeine doses administered in exercise research (3 to 9 mg • kg$^{-1}$) has not been associated with any harmful or negative responses (Wemple *et al.*, 1997; Millar-Stafford *et al.*, 2007; Goldstein *et al.*, 2010).

---

## 7. LOCATION OF THE PREMISES WHERE THE RESEARCH WILL BE CONDUCTED.

- Exercise Physiology Laboratory, Ground floor, Vivian Tower, Swansea University, Singleton Park.
- Trained Swansea University staff (Recreation supervisor and Laboratory technician) will provide supervision during testing.
- Indoor athletic track, Swansea University, Department of sport and physical recreation, Sport Centre, Sketty Lane, SA2 8QB, +44 (0)1792 602 400.

---

## 8. SUBJECT RISKS AND DISCOMFORTS

Participation in any form of physical activity can have some physiological consequences including: abnormal blood pressure, fainting, arrhythmias, post-exercise muscle soreness (24 to 48 hours after), and dehydration. However, all subjects will be health screened and risk stratified prior to undertaking exercise (Appendix A). In addition, subjects will be closely supervised at all times and fluids will be given to avoid dehydration problems.

Capillary blood sampling may cause some discomfort however this will be of short duration and will be reduced to a minimum.

There are no potential psychological problems recognised. Furthermore, participation in the study is voluntary and subjects will be free to withdraw at any time. Swansea University staff and postgraduate students are trained in first-aid and emergency procedures in case any unexpected medical emergency occurs during this study.

---

**9. INFORMATION SHEET AND INFORMED CONSENT**

*The submission should be specific about the type of consent that will be sought:*

Have you included a Subject Information Sheet for the participants of the study? YES (Appendix B)

Have you included a Subject Consent Form for the participants of the study? YES (Appendix C and D)

If written consent will not be obtained, explain why.

---

**10. COMPUTERS**

Are computers to be used to store data? YES

If so, is the data registered under the Data Protection Act? YES

NB: For UWS students, the answer to this question is YES, but the question has been included in order to stress the importance of adherence to the Data Protection Act in research activity

---

**11. STUDENT DECLARATION**

Please read the following declarations carefully and provide details below of any ways in which your project deviates from them. Having done this, each student listed in section 2 is required to sign where indicated.

1. I have ensured that there will be no active deception of participants.

2. I have ensured that no data will be personally identifiable.

3. I have ensured that no participant should suffer any undue physical or psychological discomfort

4. I certify that there will be no administration of potentially harmful drugs, medicines or foodstuffs.

5. I will obtain written permission from an appropriate authority before recruiting members of any outside institution as participants.

6. I certify that the participants will not experience any potentially unpleasant stimulation or deprivation.

7. I certify that any ethical considerations raised by this proposal have been discussed in detail with my supervisor.

8. I certify that the above statements are true with the following exception(s):

9. All collected data will be destroyed immediately after completion of the project.

Student signature: (include a signature for each student in research team)

Date:

---

## 12. SUPERVISOR'S DECLARATION

In the supervisor's opinion, this project (delete those that do not apply):

- Does not raise any significant issues.
- Raises some ethical issues, but I consider that appropriate steps and precautions have been taken and I have approved the proposal.
- Raises ethical issues that need to be considered by the Departmental Ethics Committee.
- Raises ethical issues such that it should not be allowed to proceed in its current form.

Supervisor's signature: Date:

---

## 13. ETHICS COMMITTEE DECISION (COMMITTEE USE ONLY)

ETHICAL APPROVAL: GRANTED REJECTED (delete as appropriate)

The ethical issues raised by this project have been considered by members of the Departmental Ethical Approval Committee who made the following comments:

.....

.....

.....

.....

.....

.....

Please ensure that you take account of these comments and prepare a revised submission that should be shown to your supervisor/ resubmitted to the Department Ethical Approval Committee (delete as appropriate).

Signed: Date:

(Chair, Departmental Ethics Advisory Committee)

**APPENDIX B**: Subject Information Sheet

**SUBJECT INFORMATION SHEET**

**Date: 11/05/2010**

Contact Details:
Carlos Penas Ruiz
Research student, Sport and Exercise Science
Swansea Universit[redacted]
Mob Phone: [redacted]
E-mail: [redacted]@swansea.ac.uk

Chris Terry
Research student, Sport and Exercise Science
[redacted]wansea University
[redacted]
[redacted]@swansea.ac.uk

**1. Study title**

Metabolic response and soccer skill performance after the ingestion of different doses of carbohydrate during a soccer-specific exercise protocol.

**2. Invitation paragraph**

The data obtained from your participation in the study will be used to understand the metabolic and performance effects of consuming sport drinks and gels during exercise. In addition, we would like to reward you with £100 worth of High5 products (www.highfive.co.uk), as a token of our appreciation for your time.

**3. What is the purpose of this study?**

The study will investigate the effects of carbohydrate supplementation during a high-intensity intermittent exercise protocol designed to replicate the activities and exercise intensity of a football match.

**4. Why have I been chosen?**

You have been asked to volunteer because you are a healthy female/male football player aged between 18 and 35 years who play football (soccer) regularly.

**5. What will happen to me if I take part?**

If you take part in the study you will be required to visit the laboratory on six different occasions. The two initial sessions will last approximately one and two hours and the four remaining sessions will last five hours each.

You will be expected to complete the following:

a) Preliminary testing: Following the completion of a health questionnaire, your height and weight will be measured. Subsequently, you will complete the familiarisation with the soccer skill part of the protocol (passing, dribbling and shooting), followed by the Multi-stage fitness test (bleep test) to estimate you maximal oxygen uptake and calculate the exercise intensity in which you will complete the main trials. In the second session, you will be familiarised with the exercise part of the protocol (90 min).

b) Four main trials: You will have to report to the lab at 8 am and after blood and urine samples have been taken, you will be given a standard breakfast. After 100 min of rest, you will perform two halves of 45 min, separated by 15 min recovery period. During the exercise part of the protocol, you will be expected to cover a 20 m distance walking, jogging, cruising, running backwards and sprinting at intensities dictated by an audio CD. You will ingest a flavoured beverage during the exercise and treatment gels at half time. Heart rate, perceived exertion will be monitored throughout. An initial and final venous blood sample will be taken together with capillary (finger prick) blood samples throughout the protocol.

**6. What are the possible disadvantages of taking part?**

The acute risks associated with exercise are very small however you may suffer post-exercise muscle soreness after exercise (24 to 48 hours). You will be closely supervised at all times and fluids will be provided to maintain normal hydration levels.

**7. What are the possible benefits of taking part?**

With your help, we will learn more about the metabolic and performance responses after carbohydrate drinks and gels ingestion and we may develop new strategies for carbohydrate use during intermittent exercise.

**8. Will my taking part in the study be kept confidential?**

All the information collected about you will be kept strictly confidential and will be used only for the purpose of the study.

**APPENDIX C:** Subject Consent Form

## SUBJECT CONSENT FORM

**Contact Details:**


Carlos Penas Ruiz
Research student, Sport and Exercise Science
Swansea University
Mob Phone: [redacted]
E-mail: [redacted]@swansea.ac.uk

Chris Terry
Research student, Sport and Exercise Science
Swansea University
[redacted]
[redacted]swansea.ac.uk


**Project Title:**

The effects of ingesting a larger dose of carbohydrate on metabolic responses during a soccer-specific exercise protocol.

**Please tick the box**

1. I confirm that I have read and understood the information sheet dated ...../...../..... (version number .....) for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that sections of any of data obtained may be looked at by responsible individuals from the University of Wales Swansea or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to these records. ☐

4. I agree to take part in the above study.

_____

Name of Subject Date Signature

_____

Name of Person taking consent Date Signature

_____

Researcher Date Signature

**APPENDIX D:** Health Questionnaire

**AHA/ACSM Health/Fitness Facility Pre-participation Screening Questionnaire.**

**Name:** .....

**Address:** .....

.....

**Tel. Number:** .....

**Emergency Contact** Name: ..... Tel No.: .....

Assess your health needs by marking all *true* statements.

***History***

You have had:

- ☐ a Heart Attack;
- ☐ Heart Surgery;
- ☐ Cardiac Catheterization;
- ☐ Coronary Angioplasty (PTCA);
- ☐ Pacemaker/implantable cardiac defibrillator/rhythm disturbance;
- ☐ Heart valve disease;
- ☐ Heart failure;
- ☐ Heart transplantation;
- ☐ Congenital heart disease.

*If you marked any of the statements in this section, consult your healthcare provider before engaging in exercise. You may need to use a facility with a* ***medically qualified staff****.*

**Cardiovascular risk factors**

- ☐ You are a man older than 45 years.
- ☐ You are a woman older than 55 years or you have had a hysterectomy or you are post-menopausal.
- ☐ You smoke.
- ☐ Your blood pressure is greater than 140/90.
- ☐ You don't know your blood pressure.
- ☐ You take blood pressure medication.
- ☐ Your blood cholesterol level is >240 mg/dL.
- ☐ You don't know your cholesterol level.
- ☐ You have a close blood relative who had a heart attack before age 55 (father or brother) or age 65 (mother or sister).
- ☐ You are diabetic or take medicine to control your blood sugar.
- ☐ You are physically inactive (i.e., you get less than 30 minutes of physical activity on at least 3 days per week).
- ☐ You are more than 20 pounds overweight.

***Symptoms* and other health issues:**

- ☐ You experience chest discomfort with exertion.
- ☐ You experience unreasonable breathlessness.
- ☐ You experience dizziness, fainting, blackouts.
- ☐ You take heart medications.
- ☐ You have musculoskeletal problems.
- ☐ You have concerns about the safety of exercise.
- ☐ You are pregnant.
- ☐ You take prescription medication(s).

*If you marked two or more of the statements in this section, you should consult your healthcare provider before engaging in exercise. You might benefit by using a facility with a* ***professionally qualified exercise staff*** *to guide your exercise program.*

☐ **None of the above is true.**

*You should be able to exercise safely without consulting your healthcare provider in almost any facility that meets your exercise program needs.*

AHA/ACSM indicates American Heart Association / American College of Sports Medicine

**APPENDIX E:** Diet records

# DIETARY RECORD
## Please read these important instructions carefully

- Please record all food and drink consumed.
- Please record the food and drink at the time eaten and not from memory at the end of the day.
- You should include all meals and snacks (including water, etc.).
- Remember to include any additions to foods (e.g. sauces, salad dressings, etc.).

## DESCRIBING FOODS AND DRINKS

1. Please give cooking methods (e.g. fried, steamed, poached, etc.).
2. Please give as many details about the food as possible:
   e.g. Brand names: Miracle margarine

   Generic names: cod fillet, diet coke

## RECORDING THE AMOUNTS OF FOOD YOU EAT

### Weighing foods

1. Initially weigh each component of your meal or snack before you begin eating and complete the "weight of item" column on the form.
2. When you have finished eating weigh any food item remaining and complete the "weight remaining" column on the form.

### Known quantities

3. Record the quantity of food identified on pre-packed food eaten and complete the "weight of item" column on the form (e.g. 220g tin of backed beans, etc.).
4. Record quantities in everyday terms (e.g. cup of infused tea, teaspoon of sugar, etc.).

***It is very important that you do not adjust what you eat or drink because you are keeping this record.***

***We are interested in your dietary intake, NOT the perfect diet!***

Name _____

Day _____ Date _____

- Please record all the food and drink you consume.
- Please record the method of cooking, type of food and quantity of food.

| | Time | Description of food/drink | | Quantity of food/drink | |
|---|---|---|---|---|---|
| | | Type of food | Cooking method | Weight of item | Weight remaining |
| Breakfast | | | | | |
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| Dinner | | | | | |
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| Snacks | | | | | |
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**APPENDIX F:** Standardised warm up protocol

## Standardised warm up protocol

### Over a 30 m distance

- **4 x 30 m Straight Jog**
- **2 x 30 m Sideways run**
- **2 x 30 m Leg cross-overs**
- **2 x 30 m Hip in/ Hip out**
- **2 x 30 m Lateral jump every 10 m**
- **2 x 30 m Progress speed run**

**5 min ball skills- Passing between players, 5 Shots, 5 Dribbles.**

**5 min self-led stretching**

**APPENDIX G:** Borg's perceived exertion Scale

**6**

**7 very, very light**

**8**

**9 very light**

**10**

**11 fairly light**

**12**

**13 somewhat hard**

**14**

**15 hard**

**16**

**17 very hard**

**18**

**19 very, very hard**

**20**

## APPENDIX H: Blood glucose raw data from pilot study ($mmol \cdot l^{-1}$)

| Palatinose | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Subject** | **Rest** | **pre** | **15** | **30** | **Half-time** | **45** | **60** | **75** | **90** |
| 1 | 5.00 | 5.60 | 4.40 | 4.00 | 4.30 | 3.30 | 3.80 | 3.20 | 3.60 |
| 2 | 4.10 | 4.10 | 4.40 | 4.70 | 5.80 | 2.70 | 4.90 | 3.90 | 3.70 |
| 3 | 4.20 | 3.40 | 4.10 | 4.60 | 5.00 | 3.30 | 3.30 | 3.60 | 4.60 |
| 4 | 4.90 | 5.70 | 3.50 | 4.80 | 4.80 | 2.80 | 3.20 | 4.30 | 4.50 |
| 5 | 3.30 | 3.30 | 2.30 | 2.60 | 2.90 | 2.90 | 2.40 | 2.50 | 3.10 |
| 6 | 4.70 | 4.70 | 2.30 | 3.40 | 3.60 | 2.70 | 2.70 | 3.50 | 2.50 |
| Mean | 4.37 | 4.47 | 3.50 | 4.02 | 4.40 | 2.95 | 3.38 | 3.50 | 3.67 |
| SD | 0.64 | 1.05 | 0.99 | 0.87 | 1.04 | 0.28 | 0.89 | 0.62 | 0.81 |

| Sport Drink (6%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Subject** | **Rest** | **pre** | **15** | **30** | **Half-time** | **45** | **60** | **75** | **90** |
| 1 | 3.70 | 3.70 | 5.00 | 4.70 | 3.80 | 2.90 | 2.80 | 3.10 | 3.60 |
| 2 | 5.30 | 3.40 | 2.80 | 4.30 | 6.10 | 2.60 | 2.50 | 2.80 | 3.40 |
| 3 | 4.20 | 2.90 | 3.30 | 2.80 | 3.40 | 3.10 | 2.30 | 2.90 | 3.10 |
| 4 | 6.80 | 4.30 | 6.80 | 6.15 | 6.05 | 4.95 | 4.30 | 5.10 | 5.40 |
| 5 | 3.60 | 3.90 | 2.90 | 3.10 | 3.10 | 3.10 | 2.80 | 2.60 | 3.10 |
| 6 | 4.40 | 3.60 | 4.50 | 4.90 | 5.10 | 3.30 | 2.90 | 3.90 | 4.90 |
| Mean | 4.67 | 3.63 | 4.22 | 4.33 | 4.59 | 3.33 | 2.93 | 3.40 | 3.92 |
| SD | 1.21 | 0.47 | 1.55 | 1.24 | 1.34 | 0.83 | 0.71 | 0.95 | 0.99 |

| Sucrose | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Subject** | **Rest** | **pre** | **15** | **30** | **Half-time** | **45** | **60** | **75** | **90** |
| 1 | 4.60 | 3.60 | 4.00 | 4.90 | 3.50 | 3.30 | 3.80 | 4.60 | 5.60 |
| 2 | 3.90 | 3.60 | 3.80 | 4.30 | 5.20 | 3.90 | 3.70 | 4.70 | 4.60 |
| 3 | 4.10 | 5.00 | 5.60 | 5.20 | 5.20 | 3.40 | 3.40 | 3.60 | 4.70 |
| 4 | 4.30 | 3.60 | 4.50 | 4.90 | 5.10 | 3.30 | 2.90 | 3.90 | 4.90 |
| 5 | 4.40 | 5.40 | 3.10 | 4.60 | 4.40 | 5.10 | 3.30 | 3.60 | 3.90 |
| 6 | 3.80 | 3.70 | 3.40 | 4.30 | 6.00 | 4.20 | 2.40 | 3.00 | 5.30 |
| Mean | 4.18 | 4.15 | 4.07 | 4.70 | 4.90 | 3.87 | 3.25 | 3.90 | 4.83 |
| SD | 0.31 | 0.82 | 0.89 | 0.36 | 0.85 | 0.71 | 0.52 | 0.65 | 0.59 |

## APPENDIX I: Study Raw data

### Blood glucose concentrations ($mmol \cdot l^{-1}$)

| PLACEBO | 1st Half samples | | | | 2nd half samples | | |
|---|---|---|---|---|---|---|---|
| | pre-SMS | 15 | 30 | 45 | 60 | 75 | post-SMS |
| 1 | 6.2 | 6.3 | 6.3 | 6.2 | 4.4 | 5.0 | 4.6 |
| 2 | 5.2 | 5.1 | 4.5 | 4.3 | 4.6 | 4.6 | 4.8 |
| 3 | 4.8 | 5.0 | 4.7 | 4.7 | 3.9 | 4.2 | 4.6 |
| 4 | 5.5 | 5.6 | 5.7 | 5.0 | 4.6 | 4.3 | 5.0 |
| 5 | 4.9 | 5.0 | 4.4 | 4.2 | 3.4 | 3.5 | 3.8 |
| 6 | 5.7 | 5.2 | 5.1 | 4.5 | 4.3 | 4.4 | 4.6 |
| 7 | 5.9 | 6.9 | 7.4 | 7.5 | 4.8 | 4.8 | 5.2 |
| 8 | 5.8 | 3.9 | 4.4 | 4.5 | 4.0 | 4.3 | 4.6 |
| 9 | 5.8 | 5.6 | 5.7 | 5.6 | 4.6 | 4.8 | 5.9 |
| 10 | 5.3 | 5.1 | 4.8 | 5.0 | 3.8 | 4.4 | 4.9 |
| 11 | 5.4 | 4.5 | 4.7 | 4.2 | 4.4 | 4.3 | 3.9 |
| 12 | 7.4 | 5.5 | 4.8 | 4.9 | 4.0 | 4.2 | 4.1 |
| 13 | 4.7 | 4.7 | 4.9 | 4.5 | 3.8 | 4.6 | 3.9 |
| 14 | 5.4 | 5.5 | 5.4 | 4.9 | 4.2 | 4.2 | 4.4 |
| Mean | 5.57 | 5.28 | 5.20 | 5.00 | 4.20 | 4.40 | 4.59 |
| SD | 0.68 | 0.74 | 0.85 | 0.91 | 0.39 | 0.36 | 0.57 |
| SEM | 0.18 | 0.20 | 0.23 | 0.24 | 0.11 | 0.10 | 0.15 |

| CHO10 | 1st Half samples | | | | 2nd half samples | | |
|---|---|---|---|---|---|---|---|
| | pre-SMS | 15 | 30 | 45 | 60 | 75 | post-SMS |
| 1 | 5.5 | 6.4 | 5.9 | 5.9 | 3.3 | 4.1 | 4.9 |
| 2 | 5.6 | 6.4 | 6.5 | 6.3 | 3.9 | 5.4 | 6.2 |
| 3 | 4.2 | 6.0 | 6.0 | 5.4 | 4.4 | 5.7 | 6.5 |
| 4 | 5.5 | 5.8 | 6.1 | 7.8 | 3.9 | 5.2 | 5.9 |
| 5 | 5.7 | 6.7 | 6.2 | 6.5 | 5.3 | 4.8 | 6.7 |
| 6 | 5.2 | 7.5 | 8.7 | 8.2 | 3.7 | 5.1 | 6.8 |
| 7 | 5.5 | 7.7 | 8.1 | 9.5 | 5.0 | 5.7 | 6.4 |
| 8 | 6.4 | 5.8 | 5.7 | 8.1 | 4.0 | 4.6 | 5.8 |
| 9 | 5.7 | 7.6 | 6.8 | 8.1 | 4.6 | 6.4 | 7.6 |
| 10 | 6.1 | 6.3 | 6.0 | 6.9 | 5.1 | 4.4 | 6.3 |
| 11 | 7.2 | 5.4 | 5.9 | 6.7 | 4.3 | 5.2 | 5.0 |
| 12 | 8.6 | 8.8 | 7.9 | 8.4 | 5.2 | 5.2 | 8.5 |
| 13 | 4.7 | 5.5 | 5.7 | 6.0 | 4.1 | 4.6 | 5.3 |
| 14 | 5.8 | 3.7 | 4.2 | 5.9 | 3.0 | 4.4 | 6.0 |
| Mean | 5.83 | 6.41 | 6.41 | 7.12 | 4.27 | 5.06 | 6.28 |
| SD | 1.06 | 1.24 | 1.15 | 1.21 | 0.71 | 0.63 | 0.97 |
| SEM | 0.28 | 0.33 | 0.31 | 0.32 | 0.19 | 0.17 | 0.26 |

| CHO6 | 1st Half samples | | | | 2nd half samples | | |
|---|---|---|---|---|---|---|---|
| | pre-SMS | 15 | 30 | 45 | 60 | 75 | post-SMS |
| 1 | 7.4 | 5.4 | 5.5 | 5.7 | 3.6 | 3.9 | 4.8 |
| 2 | 5.2 | 5.3 | 4.6 | 5.8 | 3.4 | 3.8 | 4.9 |
| 3 | 5.4 | 6.9 | 7.1 | 6.3 | 4.1 | 4.2 | 7.6 |
| 4 | 6.1 | 7.0 | 5.5 | 6.4 | 3.8 | 5.0 | 6.3 |
| 5 | 5.3 | 5.0 | 5.2 | 6.3 | 4.6 | 4.9 | 6.4 |
| 6 | 6.2 | 5.7 | 7.4 | 6.5 | 4.0 | 4.8 | 5.7 |
| 7 | 5.6 | 6.4 | 6.3 | 6.7 | 4.2 | 4.3 | 4.8 |
| 8 | 6.9 | 4.7 | 5.0 | 7.5 | 4.8 | 4.7 | 5.8 |
| 9 | 4.8 | 5.4 | 6.2 | 6.4 | 3.1 | 4.6 | 5.1 |
| 10 | 6.4 | 5.8 | 6.7 | 7.0 | 3.9 | 3.8 | 5.2 |
| 11 | 5.4 | 4.7 | 5.9 | 5.9 | 3.6 | 4.1 | 4.9 |
| 12 | 6.7 | 7.3 | 4.8 | 4.8 | 3.8 | 4.3 | 5.1 |
| 13 | 6.9 | 5.4 | 5.2 | 5.4 | 3.1 | 3.6 | 5.0 |
| 14 | 6.2 | 5.6 | 5.6 | 5.7 | 3.3 | 3.4 | 6.3 |
| Mean | 6.04 | 5.75 | 5.78 | 6.17 | 3.81 | 4.24 | 5.56 |
| SD | 0.77 | 0.84 | 0.85 | 0.69 | 0.51 | 0.50 | 0.83 |
| SEM | 0.21 | 0.22 | 0.23 | 0.18 | 0.14 | 0.13 | 0.22 |

**Blood lactate concentrations ($mmol \cdot l^{-1}$)**

| PLACEBO | 1st Half samples | | | | 2nd half samples | | |
|---|---|---|---|---|---|---|---|
| | pre-SMS | 15 | 30 | 45 | 60 | 75 | post-SMS |
| 1 | 2.4 | 9.9 | 9.9 | 8.8 | 8.8 | 8.9 | 6.1 |
| 2 | 1.1 | 7.0 | 6.4 | 5.9 | 4.8 | 5.6 | 5.9 |
| 3 | 1.1 | 5.5 | 5.2 | 4.3 | 3.6 | 3.4 | 3.1 |
| 4 | 0.6 | 6.1 | 2.7 | 4.6 | 4.3 | 3.1 | 5.4 |
| 5 | 0.8 | 10.9 | 12.3 | 12.2 | 8.9 | 9.4 | 9.9 |
| 6 | 1.0 | 3.6 | 3.5 | 4.2 | 2.6 | 3.0 | 4.2 |
| 7 | 1.0 | 11.4 | 12.2 | 12.0 | 8.0 | 12.2 | 9.0 |
| 8 | 0.9 | 4.4 | 3.3 | 3.1 | 2.2 | 3.3 | 5.1 |
| 9 | 1.0 | 3.0 | 2.8 | 2.9 | 2.8 | 2.6 | 2.4 |
| 10 | 0.6 | 7.9 | 6.0 | 4.8 | 5.6 | 7.1 | 6.7 |
| 11 | 1.0 | 12.0 | 11.4 | 9.3 | 10.6 | 9.3 | 8.7 |
| 12 | 0.9 | 8.9 | 5.2 | 5.1 | 5.0 | 4.1 | 4.5 |
| 13 | 1.8 | 3.1 | 2.9 | 2.5 | 3.2 | 2.8 | 3.2 |
| 14 | 0.8 | 10.2 | 10.1 | 10.6 | 11.1 | 9.8 | 11.3 |
| Mean | 1.07 | 7.42 | 6.71 | 6.45 | 5.82 | 6.04 | 6.11 |
| SD | 0.48 | 3.20 | 3.70 | 3.42 | 3.07 | 3.30 | 2.71 |
| SEM | 0.13 | 0.85 | 0.99 | 0.91 | 0.82 | 0.88 | 0.73 |

| CHO10 | 1st Half samples | | | | 2nd half samples | | |
|---|---|---|---|---|---|---|---|
| | pre-SMS | 15 | 30 | 45 | 60 | 75 | post-SMS |
| 1 | 1.0 | 7.4 | 7.6 | 8.1 | 7.5 | 9.3 | 9.7 |
| 2 | 1.8 | 8.1 | 7.2 | 7.9 | 6.9 | 6.6 | 4.6 |
| 3 | 0.6 | 5.4 | 5.4 | 4.8 | 4.9 | 3.7 | 3.5 |
| 4 | 1.0 | 7.0 | 6.3 | 5.7 | 6.2 | 6.3 | 5.9 |
| 5 | 1.4 | 12.9 | 12.7 | 13.5 | 13.4 | 13.0 | 13.4 |
| 6 | 0.6 | 5.7 | 4.4 | 3.4 | 2.8 | 6.2 | 4.8 |
| 7 | 0.7 | 13.5 | 10.9 | 10.0 | 9.5 | 9.4 | 9.6 |
| 8 | 0.8 | 5.8 | 8.7 | 6.5 | 6.2 | 8.1 | 11.6 |
| 9 | 0.6 | 4.7 | 4.5 | 2.7 | 5.0 | 3.5 | 3.1 |
| 10 | 0.7 | 11.5 | 12.5 | 10.5 | 7.5 | 8.9 | 8.8 |
| 11 | 1.0 | 13.2 | 13.7 | 13.0 | 9.6 | 8.6 | 7.2 |
| 12 | 1.1 | 8.4 | 7.7 | 6.4 | 7.3 | 7.1 | 6.0 |
| 13 | 1.0 | 6.2 | 6.6 | 6.4 | 6.4 | 6.1 | 7.2 |
| 14 | 1.4 | 9.3 | 6.7 | 8.8 | 7.5 | 8.4 | 7.6 |
| Mean | 0.98 | 8.51 | 8.21 | 7.69 | 7.19 | 7.51 | 7.36 |
| SD | 0.36 | 3.09 | 3.07 | 3.25 | 2.51 | 2.45 | 3.02 |
| SEM | 0.10 | 0.83 | 0.82 | 0.87 | 0.67 | 0.65 | 0.81 |

| CHO6 | 1st Half samples | | | | 2nd half samples | | |
|---|---|---|---|---|---|---|---|
| | pre-SMS | 15 | 30 | 45 | 60 | 75 | post-SMS |
| 1 | 0.5 | 6.3 | 6.9 | 7.6 | 4.2 | 5.1 | 2.5 |
| 2 | 1.3 | 7.0 | 6.3 | 6.4 | 4.2 | 4.7 | 5.1 |
| 3 | 0.7 | 5.8 | 3.2 | 2.5 | 2.1 | 2.2 | 2.1 |
| 4 | 1.0 | 9.1 | 5.4 | 5.3 | 6.7 | 3.7 | 3.9 |
| 5 | 1.7 | 14.1 | 13.9 | 14.1 | 11.9 | 13.1 | 12.7 |
| 6 | 1.1 | 3.2 | 2.4 | 2.2 | 3.1 | 3.0 | 2.5 |
| 7 | 0.9 | 8.4 | 10.2 | 9.4 | 7.9 | 7.0 | 6.1 |
| 8 | 0.7 | 7.0 | 4.8 | 4.9 | 4.2 | 4.8 | 6.5 |
| 9 | 0.4 | 4.4 | 5.0 | 4.2 | 4.7 | 3.2 | 3.1 |
| 10 | 0.4 | 11.4 | 6.9 | 7.3 | 7.0 | 7.1 | 6.8 |
| 11 | 1.3 | 12.4 | 11.9 | 10.3 | 8.0 | 5.8 | 8.0 |
| 12 | 1.0 | 9.5 | 8.5 | 7.7 | 6.4 | 3.5 | 6.9 |
| 13 | 0.8 | 3.3 | 3.2 | 3.4 | 4.6 | 1.7 | 4.0 |
| 14 | 1.0 | 8.1 | 8.4 | 9.4 | 7.2 | 7.2 | 8.1 |
| Mean | 0.91 | 7.86 | 6.93 | 6.76 | 5.87 | 5.15 | 5.59 |
| SD | 0.37 | 3.27 | 3.37 | 3.34 | 2.52 | 2.90 | 2.91 |
| SEM | 0.10 | 0.87 | 0.90 | 0.89 | 0.67 | 0.77 | 0.78 |

**Blood sodium concentrations ($mmol \cdot l^{-1}$)**

| PLACEBO | 1st Half samples | | | | 2nd half samples | | |
|---|---|---|---|---|---|---|---|
| | pre-SMS | 15 | 30 | 45 | 60 | 75 | post-SMS |
| 1 | 139 | 142 | 143 | 141 | 143 | 145 | 140 |
| 2 | 140 | 140 | 138 | 142 | 139 | 144 | 143 |
| 3 | 138 | 138 | 143 | 142 | 140 | 140 | 136 |
| 4 | 136 | 138 | 141 | 141 | 139 | 141 | 140 |
| 5 | 138 | 140 | 142 | 140 | 140 | 140 | 142 |
| 6 | 136 | 138 | 140 | 138 | 141 | 142 | 139 |
| 7 | 136 | 144 | 152 | 151 | 150 | 148 | 139 |
| 8 | 139 | 144 | 142 | 142 | 143 | 143 | 143 |
| 9 | 139 | 141 | 142 | 140 | 142 | 139 | 139 |
| 10 | 133 | 140 | 141 | 140 | 141 | 143 | 141 |
| 11 | 136 | 139 | 139 | 137 | 138 | 139 | 138 |
| 12 | 135 | 136 | 139 | 136 | 137 | 136 | 136 |
| 13 | 140 | 139 | 140 | 143 | 139 | 140 | 141 |
| 14 | 136 | 139 | 140 | 137 | 140 | 137 | 143 |
| Mean | 137.21 | 139.86 | 141.57 | 140.71 | 140.86 | 141.21 | 140.00 |
| SD | 2.08 | 2.28 | 3.37 | 3.67 | 3.16 | 3.21 | 2.35 |
| SEM | 0.56 | 0.61 | 0.90 | 0.98 | 0.84 | 0.86 | 0.63 |

| CHO10 | 1st Half samples | | | | 2nd half samples | | |
|---|---|---|---|---|---|---|---|
| | pre-SMS | 15 | 30 | 45 | 60 | 75 | post-SMS |
| 1 | 141 | 143 | 144 | 143 | 148 | 144 | 145 |
| 2 | 140 | 142 | 147 | 151 | 144 | 148 | 141 |
| 3 | 139 | 141 | 142 | 144 | 144 | 143 | 141 |
| 4 | 139 | 141 | 142 | 144 | 146 | 143 | 145 |
| 5 | 139 | 139 | 143 | 144 | 140 | 142 | 145 |
| 6 | 134 | 140 | 140 | 136 | 142 | 141 | 141 |
| 7 | 141 | 143 | 148 | 144 | 147 | 147 | 145 |
| 8 | 143 | 146 | 145 | 142 | 145 | 147 | 147 |
| 9 | 138 | 144 | 146 | 144 | 147 | 148 | 142 |
| 10 | 140 | 143 | 145 | 146 | 147 | 148 | 144 |
| 11 | 135 | 146 | 146 | 147 | 144 | 146 | 143 |
| 12 | 138 | 141 | 145 | 146 | 143 | 143 | 142 |
| 13 | 139 | 141 | 143 | 146 | 146 | 143 | 142 |
| 14 | 134 | 140 | 142 | 148 | 142 | 140 | 143 |
| Mean | 138.57 | 142.14 | 144.14 | 144.64 | 144.64 | 144.50 | 143.29 |
| SD | 2.65 | 2.14 | 2.25 | 3.39 | 2.34 | 2.77 | 1.90 |
| SEM | 0.71 | 0.57 | 0.60 | 0.91 | 0.63 | 0.74 | 0.51 |

| CHO6 | 1st Half samples | | | | 2nd half samples | | |
|---|---|---|---|---|---|---|---|
| | pre-SMS | 15 | 30 | 45 | 60 | 75 | post-SMS |
| 1 | 137 | 145 | 144 | 140 | 141 | 146 | 146 |
| 2 | 133 | 140 | 141 | 139 | 139 | 142 | 142 |
| 3 | 138 | 143 | 142 | 141 | 144 | 140 | 144 |
| 4 | 136 | 142 | 141 | 140 | 145 | 145 | 141 |
| 5 | 139 | 140 | 143 | 145 | 144 | 144 | 143 |
| 6 | 140 | 142 | 143 | 144 | 146 | 147 | 145 |
| 7 | 138 | 138 | 139 | 141 | 141 | 141 | 141 |
| 8 | 138 | 145 | 145 | 143 | 142 | 138 | 139 |
| 9 | 135 | 142 | 141 | 142 | 150 | 142 | 142 |
| 10 | 132 | 141 | 140 | 142 | 142 | 142 | 143 |
| 11 | 143 | 144 | 144 | 142 | 144 | 144 | 143 |
| 12 | 139 | 141 | 147 | 145 | 145 | 149 | 145 |
| 13 | 140 | 139 | 141 | 140 | 144 | 143 | 143 |
| 14 | 137 | 143 | 146 | 142 | 144 | 142 | 143 |
| Mean | 137.50 | 141.79 | 142.64 | 141.86 | 143.64 | 143.21 | 142.86 |
| SD | 2.88 | 2.12 | 2.34 | 1.88 | 2.65 | 2.89 | 1.83 |
| SEM | 0.77 | 0.57 | 0.63 | 0.50 | 0.71 | 0.77 | 0.49 |

## Blood haemoglobin concentrations ($mg \cdot l^{-1}$)

| PLACEBO | 1st Half samples | | | | 2nd half samples | | |
|---|---|---|---|---|---|---|---|
| | pre-SMS | 15 | 30 | 45 | 60 | 75 | post-SMS |
| 1 | 155 | 156 | 150 | 145 | 148 | 144 | 140 |
| 2 | 153 | 154 | 148 | 133 | 145 | 146 | 141 |
| 3 | 134 | 148 | 157 | 156 | 137 | 132 | 136 |
| 4 | 141 | 150 | 146 | 146 | 148 | 157 | 136 |
| 5 | 131 | 149 | 152 | 141 | 146 | 141 | 131 |
| 6 | 158 | 154 | 158 | 168 | 158 | 152 | 142 |
| 7 | 142 | 156 | 153 | 150 | 141 | 149 | 149 |
| 8 | 132 | 135 | 137 | 137 | 140 | 138 | 129 |
| 9 | 172 | 154 | 161 | 165 | 170 | 148 | 137 |
| 10 | 132 | 146 | 150 | 144 | 137 | 137 | 135 |
| 11 | 140 | 159 | 150 | 151 | 141 | 145 | 135 |
| 12 | 169 | 162 | 151 | 161 | 155 | 153 | 147 |
| 13 | 168 | 158 | 162 | 166 | 141 | 156 | 158 |
| 14 | 163 | 148 | 154 | 140 | 151 | 141 | 145 |
| Mean | 149.29 | 152.07 | 152.07 | 150.21 | 147.00 | 145.64 | 140.07 |
| SD | 15.04 | 6.79 | 6.43 | 11.36 | 9.17 | 7.41 | 7.73 |
| SEM | 4.02 | 1.81 | 1.72 | 3.04 | 2.45 | 1.98 | 2.07 |

| CHO10 | 1st Half samples | | | | 2nd half samples | | |
|---|---|---|---|---|---|---|---|
| | pre-SMS | 15 | 30 | 45 | 60 | 75 | post-SMS |
| 1 | 141 | 143 | 144 | 143 | 148 | 144 | 145 |
| 2 | 140 | 142 | 147 | 151 | 144 | 148 | 141 |
| 3 | 139 | 141 | 142 | 144 | 144 | 143 | 141 |
| 4 | 139 | 141 | 142 | 144 | 146 | 143 | 145 |
| 5 | 139 | 139 | 143 | 144 | 140 | 142 | 145 |
| 6 | 134 | 140 | 140 | 136 | 142 | 141 | 141 |
| 7 | 141 | 143 | 148 | 144 | 147 | 147 | 145 |
| 8 | 143 | 146 | 145 | 142 | 145 | 147 | 147 |
| 9 | 138 | 144 | 146 | 144 | 147 | 148 | 142 |
| 10 | 140 | 143 | 145 | 146 | 147 | 148 | 144 |
| 11 | 135 | 146 | 146 | 147 | 144 | 146 | 143 |
| 12 | 138 | 141 | 145 | 146 | 143 | 143 | 142 |
| 13 | 139 | 141 | 143 | 146 | 146 | 143 | 142 |
| 14 | 134 | 140 | 142 | 148 | 142 | 140 | 143 |
| Mean | 138.57 | 142.14 | 144.14 | 144.64 | 144.64 | 144.50 | 143.29 |
| SD | 2.65 | 2.14 | 2.25 | 3.39 | 2.34 | 2.77 | 1.90 |
| SEM | 0.71 | 0.57 | 0.60 | 0.91 | 0.63 | 0.74 | 0.51 |

| CHO6 | 1st Half samples | | | | 2nd half samples | | |
|---|---|---|---|---|---|---|---|
| | pre-SMS | 15 | 30 | 45 | 60 | 75 | post-SMS |
| 1 | 137 | 145 | 144 | 140 | 141 | 146 | 146 |
| 2 | 133 | 140 | 141 | 139 | 139 | 142 | 142 |
| 3 | 138 | 143 | 142 | 141 | 144 | 140 | 144 |
| 4 | 136 | 142 | 141 | 140 | 145 | 145 | 141 |
| 5 | 139 | 140 | 143 | 145 | 144 | 144 | 143 |
| 6 | 140 | 142 | 143 | 144 | 146 | 147 | 145 |
| 7 | 138 | 138 | 139 | 141 | 141 | 141 | 141 |
| 8 | 138 | 145 | 145 | 143 | 142 | 138 | 139 |
| 9 | 135 | 142 | 141 | 142 | 150 | 142 | 142 |
| 10 | 132 | 141 | 140 | 142 | 142 | 142 | 143 |
| 11 | 143 | 144 | 144 | 142 | 144 | 144 | 143 |
| 12 | 139 | 141 | 147 | 145 | 145 | 149 | 145 |
| 13 | 140 | 139 | 141 | 140 | 144 | 143 | 143 |
| 14 | 137 | 143 | 146 | 142 | 144 | 142 | 143 |
| Mean | 137.50 | 141.79 | 142.64 | 141.86 | 143.64 | 143.21 | 142.86 |
| SD | 2.88 | 2.12 | 2.34 | 1.88 | 2.65 | 2.89 | 1.83 |
| SEM | 0.77 | 0.57 | 0.63 | 0.50 | 0.71 | 0.77 | 0.49 |

## GEM 3000 Haematocrit (%)

| PLACEBO | 1st Half samples | | | | 2nd half samples | | |
|---|---|---|---|---|---|---|---|
| | pre-SMS | 15 | 30 | 45 | 60 | 75 | post-SMS |
| 1 | 46 | 48 | 48 | 50 | 48 | 51 | 50 |
| 2 | 47 | 48 | 53 | 50 | 48 | 50 | 45 |
| 3 | 53 | 51 | 53 | 51 | 49 | 50 | 50 |
| 4 | 46 | 51 | 50 | 52 | 51 | 52 | 52 |
| 5 | 44 | 49 | 49 | 49 | 49 | 45 | 49 |
| 6 | 51 | 46 | 47 | 50 | 50 | 50 | 46 |
| 7 | 51 | 56 | 45 | 46 | 50 | 48 | 51 |
| 8 | 43 | 47 | 46 | 47 | 48 | 46 | 49 |
| 9 | 49 | 50 | 50 | 50 | 52 | 51 | 50 |
| 10 | 46 | 45 | 46 | 47 | 46 | 46 | 46 |
| 11 | 47 | 56 | 48 | 51 | 49 | 47 | 51 |
| 12 | 50 | 58 | 56 | 54 | 55 | 53 | 52 |
| 13 | 47 | 45 | 47 | 50 | 47 | 49 | 54 |
| 14 | 51 | 51 | 48 | 52 | 49 | 46 | 49 |
| Mean | 47.93 | 50.07 | 49.00 | 49.93 | 49.36 | 48.86 | 49.57 |
| SD | 2.95 | 4.14 | 3.14 | 2.16 | 2.24 | 2.54 | 2.53 |
| SEM | 0.79 | 1.11 | 0.84 | 0.58 | 0.60 | 0.68 | 0.68 |

| CHO10 | 1st Half samples | | | | 2nd half samples | | |
|---|---|---|---|---|---|---|---|
| | pre-SMS | 15 | 30 | 45 | 60 | 75 | post-SMS |
| 1 | 46 | 50 | 50 | 52 | 50 | 50 | 50 |
| 2 | 48 | 53 | 50 | 51 | 53 | 53 | 52 |
| 3 | 52 | 51 | 50 | 46 | 46 | 46 | 46 |
| 4 | 49 | 52 | 52 | 51 | 52 | 55 | 55 |
| 5 | 48 | 51 | 52 | 50 | 53 | 49 | 53 |
| 6 | 47 | 51 | 47 | 50 | 49 | 48 | 48 |
| 7 | 51 | 56 | 52 | 50 | 53 | 52 | 51 |
| 8 | 49 | 54 | 51 | 50 | 53 | 51 | 56 |
| 9 | 51 | 56 | 48 | 50 | 48 | 49 | 50 |
| 10 | 49 | 52 | 53 | 53 | 50 | 52 | 54 |
| 11 | 51 | 52 | 52 | 52 | 52 | 50 | 53 |
| 12 | 50 | 53 | 57 | 55 | 53 | 56 | 53 |
| 13 | 47 | 47 | 49 | 50 | 53 | 46 | 47 |
| 14 | 53 | 50 | 50 | 47 | 49 | 48 | 51 |
| Mean | 49.36 | 52.00 | 50.93 | 50.50 | 51.00 | 50.36 | 51.36 |
| SD | 2.06 | 2.39 | 2.43 | 2.24 | 2.32 | 3.03 | 2.95 |
| SEM | 0.55 | 0.64 | 0.65 | 0.60 | 0.62 | 0.81 | 0.79 |

| CHO6 | 1st Half samples | | | | 2nd half samples | | |
|---|---|---|---|---|---|---|---|
| | pre-SMS | 15 | 30 | 45 | 60 | 75 | post-SMS |
| 1 | 45 | 47 | 47 | 47 | 46 | 47 | 53 |
| 2 | 47 | 49 | 53 | 49 | 53 | 47 | 47 |
| 3 | 47 | 53 | 52 | 52 | 51 | 50 | 51 |
| 4 | 45 | 55 | 54 | 52 | 50 | 54 | 50 |
| 5 | 42 | 51 | 50 | 52 | 51 | 49 | 57 |
| 6 | 47 | 53 | 47 | 52 | 53 | 51 | 50 |
| 7 | 47 | 49 | 51 | 53 | 51 | 49 | 50 |
| 8 | 47 | 52 | 49 | 52 | 53 | 52 | 52 |
| 9 | 51 | 53 | 54 | 55 | 55 | 50 | 49 |
| 10 | 51 | 51 | 51 | 49 | 49 | 49 | 49 |
| 11 | 50 | 57 | 50 | 48 | 48 | 48 | 48 |
| 12 | 52 | 50 | 56 | 52 | 52 | 55 | 51 |
| 13 | 45 | 53 | 49 | 48 | 49 | 44 | 49 |
| 14 | 48 | 52 | 52 | 50 | 53 | 50 | 47 |
| Mean | 47.43 | 51.79 | 51.07 | 50.79 | 51.00 | 49.64 | 50.21 |
| SD | 2.79 | 2.58 | 2.64 | 2.29 | 2.42 | 2.84 | 2.61 |
| SEM | 0.75 | 0.69 | 0.71 | 0.61 | 0.65 | 0.76 | 0.70 |

| PLACEBO | Morning BM (kg) | Pre-SMS BM (kg) | Post-SMS BM (kg) | 2% BM loss (kg) | Beverage ingested (l) | Total fluid ingested (l) | Height (cm) |
|---|---|---|---|---|---|---|---|
| 1 | 79.3 | 79.4 | 78.7 | 1.6 | 1.67 | 1.91 | 188.8 |
| 2 | 70.4 | 70.1 | 70.6 | 1.4 | 1.48 | 1.72 | 171.4 |
| 3 | 63.9 | 64.3 | 64.2 | 1.3 | 1.34 | 1.58 | 170.4 |
| 4 | 94.9 | 94.0 | 93.8 | 1.9 | 1.99 | 2.23 | 179.4 |
| 5 | 75.7 | 75.4 | 75.2 | 1.5 | 1.59 | 1.83 | 172.7 |
| 6 | 74.1 | 74.5 | 74.3 | 1.5 | 1.56 | 1.80 | 181.7 |
| 7 | 71.9 | 72.2 | 71.8 | 1.4 | 1.51 | 1.75 | 184.2 |
| 8 | 78.0 | 78.7 | 79.0 | 1.6 | 1.64 | 1.88 | 185.7 |
| 9 | 81.2 | 81.7 | 81.4 | 1.6 | 1.71 | 1.95 | 185.6 |
| 10 | 74.3 | 74.7 | 74.5 | 1.5 | 1.56 | 1.80 | 185.7 |
| 11 | 85.3 | 85.5 | 85.6 | 1.7 | 1.79 | 2.03 | 176.0 |
| 12 | 92.2 | 93.0 | 92.8 | 1.8 | 1.94 | 2.18 | 181.9 |
| 13 | 81.1 | 81.4 | 81.1 | 1.6 | 1.70 | 1.94 | 182.1 |
| 14 | 87.1 | 87.9 | 88.0 | 1.7 | 1.83 | 2.07 | 192.3 |
| Mean | 79.2 | 79.5 | 79.4 | 1.6 | 1.66 | 1.904 | 181.3 |
| SD | 8.6 | 8.5 | 8.5 | 0.2 | 0.18 | 0.18 | 6.6 |
| SEM | 2.3 | 2.3 | 2.3 | 0.0 | 0.05 | 0.05 | 1.8 |

**Participant's Anthropometry**

| CHO10 | Morning BM (kg) | Pre-SMS BM (kg) | Post-SMS BM (kg) | 2% BM loss (kg) | Beverage ingested (l) | Total fluid ingested (l) | Height (cm) |
|---|---|---|---|---|---|---|---|
| 1 | 79.8 | 79.7 | 79.4 | 1.6 | 1.7 | 1.92 | 188.8 |
| 2 | 69.9 | 70.2 | 70.5 | 1.4 | 1.5 | 1.71 | 171.2 |
| 3 | 62.0 | 62.2 | 62.4 | 1.2 | 1.3 | 1.54 | 170.3 |
| 4 | 94.1 | 93.4 | 93.5 | 1.9 | 2.0 | 2.22 | 180.1 |
| 5 | 75.4 | 75.1 | 75.2 | 1.5 | 1.6 | 1.82 | 172.4 |
| 6 | 73.7 | 74.2 | 73.9 | 1.5 | 1.5 | 1.79 | 182.5 |
| 7 | 71.5 | 71.4 | 71.2 | 1.4 | 1.5 | 1.74 | 184.2 |
| 8 | 79.3 | 79.7 | 79.6 | 1.6 | 1.7 | 1.91 | 185.7 |
| 9 | 82.4 | 82.7 | 82.9 | 1.6 | 1.7 | 1.97 | 184.9 |
| 10 | 74.8 | 74.8 | 74.3 | 1.5 | 1.6 | 1.81 | 185.2 |
| 11 | 84.4 | 84.9 | 85.3 | 1.7 | 1.8 | 2.01 | 176.2 |
| 12 | 92.5 | 92.4 | 92.1 | 1.9 | 1.9 | 2.18 | 182.8 |
| 13 | 81.9 | 82.1 | 81.6 | 1.6 | 1.7 | 1.96 | 182.2 |
| 14 | 86.2 | 86.8 | 87.0 | 1.7 | 1.8 | 2.1 | 193.0 |
| Mean | 79.1 | 79.3 | 79.2 | 1.6 | 1.66 | 1.90 | 181.4 |
| SD | 8.8 | 8.7 | 8.7 | 0.2 | 0.18 | 0.18 | 6.7 |
| SEM | 2.3 | 2.3 | 2.3 | 0.0 | 0.05 | 0.05 | 1.8 |

| CHO6 | Morning BM (kg) | Pre-SMS BM (kg) | Post-SMS BM (kg) | 2% BM loss (kg) | Beverage ingested (l) | Total fluid ingested (l) | Height (cm) |
|---|---|---|---|---|---|---|---|
| 1 | 81.0 | 80.8 | 80.6 | 1.6 | 1.7 | 1.9 | 188.6 |
| 2 | 70.4 | 70.3 | 70.5 | 1.4 | 1.5 | 1.7 | 170.3 |
| 3 | 64.0 | 64.0 | 64.4 | 1.3 | 1.3 | 1.6 | 169.8 |
| 4 | 94.3 | 94.3 | 94.1 | 1.9 | 2.0 | 2.2 | 179.8 |
| 5 | 72.5 | 72.8 | 72.9 | 1.5 | 1.5 | 1.8 | 172.3 |
| 6 | 73.4 | 73.9 | 73.9 | 1.5 | 1.5 | 1.8 | 181.1 |
| 7 | 72.1 | 72.6 | 72.2 | 1.4 | 1.5 | 1.8 | 184.6 |
| 8 | 78.1 | 78.6 | 78.9 | 1.6 | 1.6 | 1.9 | 186.1 |
| 9 | 80.3 | 80.9 | 81.0 | 1.6 | 1.7 | 1.9 | 185.2 |
| 10 | 73.8 | 74.3 | 74.1 | 1.5 | 1.5 | 1.8 | 186.4 |
| 11 | 84.6 | 84.7 | 85.0 | 1.7 | 1.8 | 2.0 | 175.9 |
| 12 | 90.2 | 90.3 | 90.4 | 1.8 | 1.9 | 2.1 | 182.4 |
| 13 | 82.5 | 82.4 | 82.0 | 1.7 | 1.7 | 2.0 | 181.7 |
| 14 | 87.4 | 87.3 | 87.7 | 1.7 | 1.8 | 2.1 | 192.2 |
| Mean | 78.9 | 79.1 | 79.1 | 1.6 | 1.66 | 1.90 | 181.2 |
| SD | 8.4 | 8.4 | 8.3 | 0.2 | 0.18 | 0.18 | 6.9 |
| SEM | 2.3 | 2.2 | 2.2 | 0.0 | 0.05 | 0.05 | 1.8 |

**Dietary intake in the Placebo trial**

| Subject | Energy | Protein | Fat | Carbohydrate | Sugars | Starch | Fibre | Calcium | Sodium | Cholesterol | Alcohol | Water |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | kcal | g | g | g | g | g | g | Mg | mg | mg | g | g |
| 1 | 2415.74 | 84.56 | 92.5 | 330.64 | 240.71 | 89.36 | 5.7 | 974.1 | 1444.4 | 691.44 | 0 | 2265.49 |
| 2 | 1780.85 | 102.54 | 88.28 | 153.42 | 13.42 | 140.21 | 4.03 | 318.7 | 4013.4 | 277.92 | 0 | 3122.69 |
| 3 | 1218 | 42.51 | 58.76 | 138.69 | 55.21 | 83.68 | 12.19 | 754.7 | 3277 | 319.2 | 0 | 573.34 |
| 4 | 1386.15 | 58.88 | 75.06 | 126.59 | 49.74 | 76.48 | 8.02 | 947.65 | 2840.45 | 123.3 | 0 | 1674.38 |
| 5 | 1386.15 | 58.88 | 75.06 | 126.59 | 49.74 | 76.48 | 8.02 | 947.65 | 2840.45 | 123.3 | 0 | 1674.38 |
| 6 | 2102.7 | 91.4 | 97.38 | 229.11 | 47.97 | 180.53 | 11.69 | 499.9 | 3583.4 | 268.1 | 0 | 1054.37 |
| 7 | 1978.95 | 82.51 | 70.04 | 271.72 | 76.83 | 193.2 | 11.35 | 360.56 | 3450.3 | 231.15 | 0 | 488.28 |
| 8 | 1319.84 | 46.28 | 54.77 | 148.08 | 63.77 | 84.31 | 3.54 | 824.42 | 1911.5 | 105.19 | 12 | 1650.52 |
| 9 | 1550.51 | 76.84 | 58.84 | 190.47 | 21.37 | 168.01 | 14.63 | 474 | 2518.7 | 549.8 | 0 | 610.56 |
| 10 | 1254.09 | 49.22 | 38.02 | 187.4 | 26.34 | 160.49 | 10.95 | 580.96 | 2143.95 | 108.4 | 0 | 1521.78 |
| 11 | 1709.75 | 53.85 | 77.48 | 212.64 | 40.28 | 171.36 | 11.99 | 733.65 | 2047.65 | 87.42 | 0 | 349.82 |
| 12 | 1236.5 | 57.02 | 36.07 | 181.92 | 12.14 | 155.28 | 10.95 | 456.6 | 2027.5 | 98.1 | 0 | 1993.1 |
| 13 | 1477.17 | 70.67 | 63.68 | 200.01 | 95.35 | 104.55 | 8.75 | 360.65 | 1682.5 | 82.12 | 0 | 3319.58 |
| 14 | 2268.11 | 58.92 | 89.11 | 327.87 | 153.04 | 172.05 | 12.6 | 971.88 | 2888.25 | 165.16 | 0 | 713.73 |
| Mean | 1,648.89 | 66.72 | 69.65 | 201.80 | 67.57 | 132.57 | 9.60 | 657.53 | 2,619.25 | 230.76 | 0.86 | 1,500.86 |
| SD | 402.36 | 18.25 | 19.13 | 67.65 | 61.96 | 44.13 | 3.36 | 249.05 | 776.50 | 184.77 | 3.21 | 947.47 |
| SEM | 107.53 | 4.88 | 5.11 | 18.08 | 16.56 | 11.79 | 0.90 | 66.56 | 207.53 | 49.38 | 0.86 | 253.22 |

**Dietary intake in the CHO10 trial**

| Subject | Energy kcal | Protein g | Fat g | Carbohydrate g | Sugars g | Starch g | Fibre g | Calcium Mg | Sodium mg | Cholesterol mg | Alcohol g | Water g |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 1803.00 | 75.36 | 62.25 | 251.13 | 68.40 | 167.00 | 4.06 | 767.10 | 3679.70 | 134.30 | 0.00 | 1482.16 |
| **2** | 1922.67 | 80.49 | 57.51 | 288.98 | 132.93 | 151.02 | 9.53 | 736.96 | 1876.03 | 143.80 | 0.00 | 2444.93 |
| **3** | 1596.51 | 66.61 | 59.61 | 211.75 | 34.55 | 176.20 | 11.84 | 718.21 | 3302.38 | 101.36 | 0.00 | 475.87 |
| **4** | 1159.88 | 39.29 | 47.96 | 152.22 | 85.13 | 67.03 | 9.42 | 734.71 | 1685.15 | 101.33 | 0.00 | 2397.17 |
| **5** | 1550.21 | 62.41 | 54.36 | 217.95 | 55.89 | 159.84 | 8.75 | 468.40 | 2590.10 | 280.82 | 0.00 | 3259.39 |
| **6** | 1600.20 | 60.42 | 68.41 | 197.94 | 66.36 | 130.31 | 6.83 | 597.10 | 2265.93 | 123.38 | 0.00 | 2104.15 |
| **7** | 2134.99 | 101.27 | 79.34 | 270.53 | 55.92 | 214.08 | 9.34 | 1285.13 | 2764.94 | 604.35 | 0.00 | 2248.38 |
| **8** | 2817.88 | 91.56 | 98.38 | 405.01 | 146.85 | 258.16 | 9.42 | 1045.80 | 2890.76 | 412.24 | 7.26 | 3451.00 |
| **9** | 1748.78 | 127.49 | 52.89 | 203.31 | 84.66 | 118.66 | 6.97 | 1107.06 | 2402.65 | 239.40 | 0.00 | 511.16 |
| **10** | 1295.31 | 55.92 | 83.82 | 82.88 | 9.04 | 73.65 | 5.22 | 979.01 | 2113.26 | 184.52 | 0.00 | 957.46 |
| **11** | 1164.85 | 63.75 | 58.36 | 102.52 | 6.02 | 95.66 | 10.30 | 113.75 | 1211.25 | 156.20 | 0.00 | 372.22 |
| **12** | 1534.26 | 54.34 | 50.03 | 231.23 | 119.83 | 111.46 | 4.89 | 331.04 | 1689.50 | 144.92 | 0.00 | 1255.35 |
| **13** | 1551.01 | 43.69 | 74.68 | 187.72 | 90.72 | 97.07 | 6.40 | 315.35 | 1306.60 | 47.84 | 0.00 | 2199.04 |
| **14** | 1626.48 | 75.08 | 54.71 | 222.43 | 97.10 | 120.99 | 7.47 | 763.54 | 2374.85 | 184.59 | 0.00 | 3018.69 |
| **Mean** | 1679.00 | 71.26 | 64.45 | 216.11 | 75.24 | 138.65 | 7.89 | 711.65 | 2296.65 | 204.22 | 0.52 | 1869.78 |
| **SD** | 423.84 | 23.48 | 14.66 | 79.18 | 42.11 | 53.52 | 2.27 | 328.99 | 718.57 | 146.18 | 1.94 | 1039.92 |
| **SEM** | 113.28 | 6.28 | 3.92 | 21.16 | 11.25 | 14.30 | 0.61 | 87.93 | 192.05 | 39.07 | 0.52 | 277.93 |

**Dietary intake in the CHO6 trial**

| Subject | Energy | Protein | Fat | Carbohydrate | Sugars | Starch | Fibre | Calcium | Sodium | Cholesterol | Alcohol | Water |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | kcal | g | g | g | g | g | g | Mg | mg | mg | g | g |
| 1 | 1703.94 | 103.02 | 63.62 | 191.21 | 72.08 | 119.13 | 11.02 | 791.87 | 1892.99 | 242.11 | 0.00 | 1345.47 |
| 2 | 2093.28 | 85.31 | 77.21 | 280.66 | 160.02 | 119.22 | 11.96 | 1035.58 | 1749.84 | 258.96 | 0.00 | 3474.61 |
| 3 | 1264.00 | 72.18 | 56.40 | 124.60 | 55.56 | 67.68 | 8.22 | 476.20 | 2137.40 | 181.16 | 0.00 | 666.98 |
| 4 | 1589.07 | 70.96 | 68.20 | 184.12 | 60.84 | 122.64 | 5.53 | 621.12 | 2350.40 | 223.02 | 0.00 | 2131.75 |
| 5 | 1236.42 | 74.25 | 46.74 | 138.55 | 36.07 | 98.26 | 5.41 | 128.60 | 1288.79 | 186.42 | 0.00 | 2407.66 |
| 6 | 2037.24 | 86.63 | 75.50 | 269.56 | 137.41 | 129.50 | 16.47 | 490.59 | 1728.60 | 115.88 | 0.00 | 2181.02 |
| 7 | 1588.84 | 72.20 | 57.03 | 209.61 | 55.13 | 154.54 | 10.95 | 542.65 | 1470.88 | 377.57 | 0.00 | 994.23 |
| 8 | 2306.50 | 109.36 | 118.06 | 215.56 | 82.73 | 132.21 | 17.04 | 1317.28 | 3676.44 | 261.30 | 0.00 | 2281.30 |
| 9 | 1346.50 | 100.64 | 35.98 | 150.56 | 12.89 | 135.67 | 9.04 | 402.02 | 1693.90 | 280.56 | 0.00 | 557.94 |
| 10 | 1499.39 | 45.87 | 65.34 | 192.46 | 36.67 | 155.85 | 8.51 | 312.55 | 2547.40 | 97.94 | 0.00 | 855.15 |
| 11 | 1590.75 | 57.88 | 74.71 | 183.18 | 7.36 | 175.82 | 11.64 | 285.00 | 2928.90 | 158.60 | 0.00 | 616.05 |
| 12 | 2351.43 | 80.20 | 96.13 | 310.53 | 159.96 | 115.23 | 7.82 | 604.63 | 4582.24 | 354.72 | 0.00 | 3149.29 |
| 13 | 2148.11 | 97.84 | 74.88 | 246.29 | 82.25 | 159.94 | 8.00 | 1403.02 | 2667.88 | 231.37 | 22.96 | 2023.15 |
| 14 | 1571.70 | 57.28 | 67.89 | 195.75 | 79.48 | 114.99 | 8.86 | 949.90 | 1438.35 | 185.70 | 0.00 | 1823.55 |
| Mean | 1737.66 | 79.54 | 69.84 | 206.62 | 74.18 | 128.62 | 10.03 | 668.64 | 2296.72 | 225.38 | 1.64 | 1750.58 |
| SD | 378.75 | 18.84 | 20.08 | 54.08 | 48.73 | 27.60 | 3.48 | 383.97 | 933.92 | 80.10 | 6.14 | 939.48 |
| SEM | 101.22 | 5.03 | 5.37 | 14.45 | 13.02 | 7.38 | 0.93 | 102.62 | 249.60 | 21.41 | 1.64 | 251.09 |

**Plasma osmolality (mosmol·kg$^{-1}$)**

| | Placebo | | CHO10 | | CHO6 | |
|---|---|---|---|---|---|---|
| **Subject** | **Pre-SMS** | **Post-SMS** | **Pre-SMS** | **Post-SMS** | **Pre-SMS** | **Post-SMS** |
| **1** | 0.301 | 0.326 | 0.292 | 0.306 | 0.297 | 0.298 |
| **2** | 0.293 | 0.288 | 0.271 | 0.270 | 0.286 | 0.295 |
| **3** | 0.292 | 0.282 | 0.300 | 0.314 | 0.293 | 0.309 |
| **4** | 0.293 | 0.296 | 0.310 | 0.303 | 0.279 | 0.278 |
| **5** | 0.311 | 0.301 | 0.303 | 0.299 | 0.282 | 0.296 |
| **6** | 0.285 | 0.291 | 0.302 | 0.296 | 0.293 | 0.273 |
| **7** | 0.311 | 0.306 | 0.282 | 0.298 | 0.294 | 0.306 |
| **8** | 0.293 | 0.308 | 0.290 | 0.301 | 0.290 | 0.294 |
| **9** | 0.296 | 0.302 | 0.299 | 0.302 | 0.283 | 0.289 |
| **10** | 0.277 | 0.279 | 0.275 | 0.286 | 0.296 | 0.298 |
| **11** | 0.276 | 0.272 | 0.290 | 0.299 | 0.284 | 0.286 |
| **12** | 0.289 | 0.282 | 0.290 | 0.292 | 0.308 | 0.321 |
| **13** | 0.283 | 0.278 | 0.290 | 0.297 | 0.293 | 0.301 |
| **14** | 0.292 | 0.293 | 0.295 | 0.305 | 0.293 | 0.296 |
| **Mean** | 0.292 | 0.293 | 0.292 | 0.297 | 0.290 | 0.295 |
| **SD** | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| **SEM** | 0.003 | 0.004 | 0.003 | 0.003 | 0.002 | 0.003 |

**Urine osmolality (mosmol·kg$^{-1}$)**

| | Placebo | | CHO10 | | CHO6 | |
|---|---|---|---|---|---|---|
| **Subject** | **Pre-SMS** | **Post-SMS** | **Pre-SMS** | **Post-SMS** | **Pre-SMS** | **Post-SMS** |
| **1** | 1.048 | 0.664 | 0.513 | 0.15 | 0.347 | 0.147 |
| **2** | 0.704 | 0.267 | 0.693 | 0.115 | 0.498 | 0.053 |
| **3** | 0.482 | 0.193 | 0.722 | 0.733 | 0.889 | 0.222 |
| **4** | 0.763 | 0.170 | 0.713 | 0.153 | 0.496 | 0.138 |
| **5** | 0.726 | 0.481 | 0.99 | 0.702 | 0.853 | 0.712 |
| **6** | 0.946 | 0.104 | 0.969 | 0.136 | 0.944 | 0.105 |
| **7** | 0.844 | 0.488 | 1.064 | 0.298 | 0.859 | 0.206 |
| **8** | 0.958 | 0.365 | 1.074 | 0.428 | 1.025 | 0.562 |
| **9** | 0.842 | 0.509 | 0.937 | 0.439 | 0.577 | 0.238 |
| **10** | 0.819 | 0.310 | 0.722 | 0.562 | 0.968 | 0.975 |
| **11** | 0.392 | 0.201 | 0.87 | 0.489 | 0.234 | 0.464 |
| **12** | 0.840 | 0.475 | 0.951 | 0.231 | 0.874 | 0.701 |
| **13** | 0.922 | 0.525 | 0.499 | 0.562 | 0.785 | 0.701 |
| **14** | 0.905 | 0.630 | 1.076 | 0.754 | 0.763 | 0.17 |
| **Mean** | 0.799 | 0.384 | 0.8424 | 0.4109 | 0.7223 | 0.3853 |
| **SD** | 0.180 | 0.179 | 0.1983 | 0.2324 | 0.2473 | 0.2942 |
| **SEM** | 0.048 | 0.048 | 0.0530 | 0.0621 | 0.0661 | 0.0786 |

**Heart rate (beats·min$^{-1}$)**

| PLA | | First Half | | | Half-Time | Second Half | | |
|---|---|---|---|---|---|---|---|---|
| | pre-SMS | 15 | 30 | 45 | | 60 | 75 | pos-SMS |
| 1 | 53 | 168 | 168 | 166 | 102 | 168 | 170 | 171 |
| 2 | 70 | 195 | 194 | 195 | 100 | 188 | 186 | 191 |
| 3 | 58 | 177 | 176 | 178 | 97 | 168 | 172 | 175 |
| 4 | 61 | 170 | 161 | 172 | 94 | 170 | 168 | 174 |
| 5 | 55 | 171 | 169 | 176 | 89 | 174 | 172 | 177 |
| 6 | 49 | 168 | 174 | 170 | 94 | 165 | 173 | 175 |
| 7 | 57 | 202 | 200 | 197 | 94 | 198 | 204 | 198 |
| 8 | 50 | 150 | 155 | 155 | 75 | 148 | 153 | 156 |
| 9 | 52 | 165 | 172 | 163 | 109 | 165 | 160 | 174 |
| 10 | 51 | 184 | 176 | 176 | 74 | 180 | 177 | 183 |
| 11 | 59 | 187 | 182 | 180 | 105 | 177 | 174 | 181 |
| 12 | 63 | 192 | 189 | 190 | 105 | 186 | 187 | 194 |
| 13 | 52 | 163 | 164 | 160 | 70 | 160 | 160 | 165 |
| 14 | 64 | 182 | 182 | 168 | 102 | 182 | 183 | 185 |
| Mean | 57 | 177 | 176 | 175 | 94 | 174 | 174 | 179 |
| SD | 6 | 14 | 13 | 13 | 12 | 13 | 13 | 11 |
| SEM | 2 | 4 | 3 | 3 | 3 | 3 | 3 | 3 |

| CHO10 | | First Half | | | Half-Time | Second Half | | |
|---|---|---|---|---|---|---|---|---|
| | pre-SMS | 15 | 30 | 45 | | 60 | 75 | pos-SMS |
| 1 | 61 | 199 | 198 | 198 | 94 | 192 | 195 | 196 |
| 2 | 61 | 176 | 184 | 180 | 90 | 181 | 178 | 182 |
| 3 | 55 | 174 | 175 | 172 | 119 | 177 | 179 | 178 |
| 4 | 52 | 180 | 177 | 179 | 95 | 184 | 182 | 187 |
| 5 | 50 | 188 | 179 | 165 | 84 | 168 | 172 | 171 |
| 6 | 51 | 202 | 200 | 198 | 119 | 199 | 199 | 201 |
| 7 | 56 | 173 | 177 | 178 | 84 | 177 | 174 | 181 |
| 8 | 52 | 170 | 171 | 174 | 86 | 168 | 168 | 165 |
| 9 | 54 | 192 | 194 | 192 | 100 | 191 | 192 | 196 |
| 10 | 66 | 188 | 188 | 187 | 109 | 183 | 176 | 183 |
| 11 | 68 | 190 | 193 | 190 | 91 | 193 | 195 | 190 |
| 12 | 57 | 171 | 176 | 175 | 88 | 177 | 177 | 180 |
| 13 | 64 | 185 | 177 | 182 | 77 | 182 | 183 | 182 |
| 14 | 57 | 182 | 183 | 181 | 94 | 181 | 182 | 183 |
| Mean | 7 | 11 | 10 | 10 | 13 | 10 | 10 | 10 |
| SD | 2 | 3 | 3 | 3 | 4 | 3 | 3 | 3 |
| SEM | 61 | 199 | 198 | 198 | 94 | 192 | 195 | 196 |

| CHO6 | | First Half | | | Half-Time | Second Half | | |
|---|---|---|---|---|---|---|---|---|
| | pre-SMS | 15 | 30 | 45 | | 60 | 75 | Post-SMS |
| 1 | 51 | 165 | 168 | 161 | 76 | 158 | 158 | 141 |
| 2 | 66 | 183 | 189 | 192 | 106 | 182 | 193 | 192 |
| 3 | 72 | 181 | 186 | 189 | 104 | 168 | 155 | 176 |
| 4 | 59 | 174 | 174 | 177 | 94 | 174 | 174 | 176 |
| 5 | 46 | 175 | 173 | 172 | 104 | 180 | 184 | 186 |
| 6 | 51 | 168 | 165 | 163 | 88 | 168 | 169 | 169 |
| 7 | 55 | 195 | 201 | 198 | 114 | 198 | 198 | 196 |
| 8 | 55 | 173 | 176 | 166 | 89 | 167 | 170 | 172 |
| 9 | 44 | 157 | 165 | 166 | 79 | 174 | 166 | 165 |
| 10 | 64 | 197 | 189 | 173 | 109 | 192 | 191 | 186 |
| 11 | 64 | 183 | 183 | 179 | 102 | 175 | 174 | 179 |
| 12 | 64 | 197 | 195 | 194 | 123 | 196 | 194 | 197 |
| 13 | 48 | 167 | 167 | 169 | 77 | 170 | 163 | 172 |
| 14 | 66 | 186 | 185 | 186 | 108 | 181 | 181 | 184 |
| Mean | 58 | 179 | 180 | 178 | 98 | 177 | 176 | 178 |
| SD | 9 | 12 | 12 | 12 | 15 | 12 | 14 | 15 |
| SEM | 2 | 3 | 3 | 3 | 4 | 3 | 4 | 4 |

## Rates of perceived exertion (Borg's scale)

| PLA | | First Half | | | Second Half | | |
|---|---|---|---|---|---|---|---|
| | pre-SMS | 15 | 30 | 45 | 60 | 75 | pos-SMS |
| 1 | 6 | 14 | 16 | 17 | 17 | 17 | 18 |
| 2 | 6 | 12 | 15 | 16 | 15 | 16 | 16 |
| 3 | 6 | 14 | 15 | 15 | 14 | 16 | 17 |
| 4 | 6 | 11 | 12 | 11 | 11 | 13 | 14 |
| 5 | 6 | 12 | 13 | 14 | 13 | 14 | 15 |
| 6 | 6 | 11 | 12 | 13 | 13 | 13 | 14 |
| 7 | 6 | 12 | 14 | 15 | 14 | 16 | 17 |
| 8 | 6 | 13 | 14 | 13 | 14 | 15 | 14 |
| 9 | 6 | 14 | 14 | 15 | 16 | 17 | 17 |
| 10 | 6 | 13 | 13 | 13 | 14 | 14 | 14 |
| 11 | 6 | 14 | 15 | 14 | 15 | 16 | 15 |
| 12 | 6 | 13 | 14 | 14 | 14 | 15 | 16 |
| 13 | 6 | 12 | 13 | 14 | 13 | 14 | 15 |
| 14 | 6 | 12 | 12 | 13 | 13 | 15 | 15 |
| Mean | 6 | 13 | 14 | 14 | 14 | 15 | 16 |
| SD | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| SEM | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| CHO10 | | First Half | | | Second Half | | |
|---|---|---|---|---|---|---|---|
| | pre-SMS | 15 | 30 | 45 | 60 | 75 | pos-SMS |
| 1 | 6 | 13 | 14 | 14 | 13 | 14 | 14 |
| 2 | 6 | 15 | 15 | 16 | 14 | 16 | 15 |
| 3 | 6 | 13 | 15 | 17 | 15 | 17 | 16 |
| 4 | 6 | 12 | 12 | 13 | 12 | 12 | 14 |
| 5 | 6 | 12 | 12 | 13 | 13 | 14 | 15 |
| 6 | 6 | 11 | 12 | 13 | 12 | 12 | 13 |
| 7 | 6 | 13 | 13 | 14 | 15 | 16 | 17 |
| 8 | 6 | 15 | 15 | 16 | 15 | 16 | 19 |
| 9 | 6 | 15 | 17 | 17 | 15 | 16 | 18 |
| 10 | 6 | 14 | 15 | 15 | 15 | 16 | 17 |
| 11 | 6 | 14 | 14 | 15 | 16 | 18 | 17 |
| 12 | 6 | 14 | 14 | 15 | 14 | 15 | 16 |
| 13 | 6 | 12 | 13 | 14 | 13 | 14 | 14 |
| 14 | 6 | 12 | 14 | 15 | 11 | 14 | 15 |
| Mean | 6 | 13 | 14 | 15 | 14 | 15 | 16 |
| SD | 0 | 1 | 1 | 1 | 1 | 2 | 2 |
| SEM | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| CHO6 | First Half | | | | Second Half | | |
|---|---|---|---|---|---|---|---|
| | pre-SMS | 15 | 30 | 45 | 60 | 75 | pos-SMS |
| 1 | 6 | 13 | 14 | 15 | 13 | 15 | 18 |
| 2 | 6 | 10 | 13 | 13 | 13 | 15 | 15 |
| 3 | 6 | 14 | 14 | 14 | 15 | 15 | 15 |
| 4 | 6 | 14 | 13 | 14 | 13 | 14 | 16 |
| 5 | 6 | 12 | 13 | 14 | 15 | 14 | 15 |
| 6 | 6 | 12 | 13 | 14 | 12 | 13 | 14 |
| 7 | 6 | 10 | 14 | 15 | 15 | 17 | 17 |
| 8 | 6 | 14 | 14 | 13 | 12 | 13 | 15 |
| 9 | 6 | 14 | 16 | 15 | 16 | 16 | 17 |
| 10 | 6 | 13 | 14 | 14 | 14 | 15 | 15 |
| 11 | 6 | 15 | 16 | 15 | 15 | 16 | 16 |
| 12 | 6 | 13 | 14 | 14 | 14 | 14 | 16 |
| 13 | 6 | 13 | 14 | 14 | 15 | 14 | 15 |
| 14 | 6 | 11 | 12 | 12 | 11 | 13 | 14 |
| Mean | 6 | 13 | 14 | 14 | 14 | 15 | 16 |
| SD | 0 | 2 | 1 | 1 | 1 | 1 | 1 |
| SEM | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Sprint speed ($m \cdot s^{-1}$)**

| PLA | First Half | | | Second Half | | |
|---|---|---|---|---|---|---|
| | 15 | 30 | 45 | 60 | 75 | pos-SMS |
| 1 | 5.75 | 5.91 | 5.83 | 5.83 | 5.81 | 5.75 |
| 2 | 5.13 | 5.03 | 4.90 | 4.85 | 4.87 | 4.90 |
| 3 | 5.45 | 5.20 | 5.00 | 5.26 | 5.07 | 4.67 |
| 4 | 6.04 | 5.76 | 5.55 | 5.84 | 5.70 | 5.80 |
| 5 | 6.11 | 6.25 | 6.21 | 6.13 | 6.21 | 6.20 |
| 6 | 5.69 | 5.76 | 5.89 | 5.67 | 5.86 | 5.94 |
| 7 | 5.60 | 5.65 | 5.58 | 5.45 | 5.69 | 5.71 |
| 8 | 5.51 | 5.82 | 5.72 | 5.55 | 5.71 | 5.88 |
| 9 | 5.71 | 5.58 | 5.63 | 5.44 | 5.91 | 5.53 |
| 10 | 5.85 | 5.77 | 5.66 | 5.78 | 5.71 | 5.70 |
| 11 | 5.14 | 4.82 | 5.01 | 4.93 | 4.92 | 4.89 |
| 12 | 5.29 | 5.39 | 5.20 | 5.16 | 5.36 | 5.44 |
| 13 | 6.03 | 5.96 | 5.86 | 5.91 | 5.98 | 5.78 |
| 14 | 5.64 | 5.61 | 5.54 | 5.52 | 5.60 | 5.55 |
| Mean | 0.33 | 0.40 | 0.40 | 0.39 | 0.42 | 0.46 |
| SD | 0.09 | 0.11 | 0.11 | 0.11 | 0.12 | 0.13 |
| SEM | 5.75 | 5.91 | 5.83 | 5.83 | 5.81 | 5.75 |

| CHO10 | First Half | | | Second Half | | |
|---|---|---|---|---|---|---|
| | 15 | 30 | 45 | 60 | 75 | pos-SMS |
| 1 | 5.75 | 5.83 | 5.82 | 5.81 | 5.95 | 6.03 |
| 2 | 5.01 | 5.31 | 5.18 | 5.22 | 5.29 | 4.90 |
| 3 | 5.82 | 5.53 | 5.63 | 5.75 | 4.99 | 5.43 |
| 4 | 6.03 | 5.96 | 5.99 | 6.03 | 6.30 | 6.13 |
| 5 | 5.95 | 5.95 | 5.93 | 5.87 | 5.99 | 6.07 |
| 6 | 5.88 | 5.74 | 5.46 | 5.64 | 5.94 | 5.72 |
| 7 | 5.62 | 5.56 | 5.58 | 5.66 | 5.64 | 5.52 |
| 8 | 5.58 | 5.90 | 5.83 | 5.76 | 5.85 | 5.79 |
| 9 | 5.95 | 5.88 | 6.05 | 5.87 | 5.85 | 5.90 |
| 10 | 5.91 | 6.03 | 6.20 | 6.06 | 6.25 | 6.00 |
| 11 | 5.60 | 5.54 | 5.46 | 5.19 | 5.40 | 5.24 |
| 12 | 5.70 | 5.76 | 5.86 | 5.92 | 5.93 | 5.95 |
| 13 | 5.94 | 5.50 | 5.52 | 5.49 | 5.49 | 5.63 |
| 14 | 5.75 | 5.73 | 5.73 | 5.71 | 5.76 | 5.72 |
| Mean | 0.27 | 0.22 | 0.29 | 0.27 | 0.38 | 0.36 |
| SD | 0.07 | 0.06 | 0.08 | 0.08 | 0.11 | 0.10 |
| SEM | 5.75 | 5.83 | 5.82 | 5.81 | 5.95 | 6.03 |

| CHO6 | First Half | | | Second Half | | |
|---|---|---|---|---|---|---|
| | 15 | 30 | 45 | 60 | 75 | pos-SMS |
| 1 | 5.72 | 5.68 | 5.79 | 5.65 | 5.76 | 5.87 |
| 2 | 5.30 | 5.30 | 5.15 | 5.08 | 5.07 | 5.03 |
| 3 | 5.56 | 5.48 | 5.37 | 4.84 | 5.05 | 5.03 |
| 4 | 5.78 | 6.02 | 5.82 | 5.82 | 5.72 | 5.90 |
| 5 | 6.03 | 6.05 | 5.99 | 5.98 | 6.10 | 6.13 |
| 6 | 5.82 | 5.85 | 5.72 | 5.94 | 6.07 | 5.95 |
| 7 | 5.76 | 5.75 | 5.66 | 5.65 | 5.69 | 5.64 |
| 8 | 5.82 | 5.81 | 5.68 | 5.92 | 5.79 | 5.72 |
| 9 | 6.09 | 5.86 | 5.77 | 5.85 | 6.07 | 6.07 |
| 10 | 5.73 | 5.68 | 5.70 | 5.69 | 5.67 | 5.63 |
| 11 | 5.18 | 5.00 | 5.44 | 5.21 | 5.34 | 5.51 |
| 12 | 5.43 | 5.51 | 5.39 | 5.39 | 5.44 | 5.46 |
| 13 | 5.75 | 5.78 | 5.86 | 5.80 | 5.63 | 5.86 |
| 14 | 5.69 | 5.68 | 5.64 | 5.60 | 5.65 | 5.68 |
| Mean | 0.26 | 0.29 | 0.24 | 0.36 | 0.34 | 0.35 |
| SD | 0.07 | 0.08 | 0.07 | 0.10 | 0.10 | 0.10 |
| SEM | 5.72 | 5.68 | 5.79 | 5.65 | 5.76 | 5.87 |

**APPENDIX J:** Comparison between Haematocric assessment methods (GEM 3000 and manual micro-centrifuged method (Bland and Altman, 1986)

| | Placebo | | | | CHO10 | | | | CHO6 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-SMS | | Post-SMS | | Pre-SMS | | Post-SMS | | Pre-SMS | | Post-SMS | |
| | GEM | Manual | GEM | Manual | GEM | Manual | GEM | Manual | GEM | Manual | GEM | Manual |
| **1** | 46 | 47 | 50 | 49 | 46 | 46 | 50 | 48 | 45 | 45 | 53 | 47 |
| **2** | 47 | 50 | 45 | 49 | 48 | 50 | 52 | 51 | 47 | 48 | 47 | 48.5 |
| **3** | 53 | 49 | 50 | 51 | 52 | 48 | 50 | 50 | 47 | 50 | 51 | 53 |
| **4** | 46 | 47 | 52 | 49.5 | 49 | 47 | 55 | 53 | 45 | 52 | 50 | 51 |
| **5** | 44 | 48 | 49 | 48 | 48 | 49 | 53 | 52 | 42 | 44 | 57 | 57 |
| **6** | 51 | 49 | 46 | 46 | 47 | 52 | 48 | 51 | 47 | 49 | 50 | 50 |
| **7** | 51 | 47 | 51 | 49 | 51 | 49.5 | 51 | 51 | 47 | 48 | 50 | 50 |
| **8** | 43 | 45 | 49 | 49 | 49 | 48 | 56 | 51 | 47 | 47 | 52 | 51 |
| **9** | 49 | 47 | 50 | 49 | 51 | 51.5 | 50 | 51 | 51 | 52 | 49 | 52.5 |
| **10** | 46 | 45 | 46 | 47 | 49 | 51 | 54 | 53 | 51 | 49 | 49 | 51 |
| **11** | 47 | 51 | 51 | 50 | 51 | 50 | 53 | 52 | 50 | 49 | 48 | 51.5 |
| **12** | 50 | 52 | 52 | 54 | 50 | 52 | 53 | 52.5 | 52 | 50 | 51 | 52 |
| **13** | 47 | 47 | 54 | 49 | 47 | 47 | 47 | 50 | 45 | 45 | 49 | 47 |
| **14** | 51 | 48 | 49 | 50 | 53 | 50 | 51 | 50 | 48 | 47 | 47 | 50 |
| **Mean** | 47.9 | 48.0 | 49.6 | 49.2 | 49.4 | 49.4 | 51.6 | 51.1 | 47.4 | 48.2 | 50.2 | 50.8 |
| **SD** | 2.9 | 2.0 | 2.5 | 1.8 | 2.1 | 1.9 | 2.6 | 1.4 | 2.8 | 2.5 | 2.6 | 2.5 |
| ***SEM*** | 0.8 | 0.5 | 0.7 | 0.5 | 0.5 | 0.5 | 0.7 | 0.4 | 0.7 | 0.7 | 0.7 | 0.7 |

**Table 7.1** Correlation coefficient between the two methods to assess haematocrit

| | |
|---|---|
| **Variable Y** | Micro_centrifuged (Log) |
| **Variable X** | GEM_3000 (Log) |
| **Sample size** | 84 |
| **Correlation coefficient r** | **0.6145** |
| **Significance level** | P<0.0001 |
| **95% Confidence interval for r** | 0.4608 to 0.7324 |

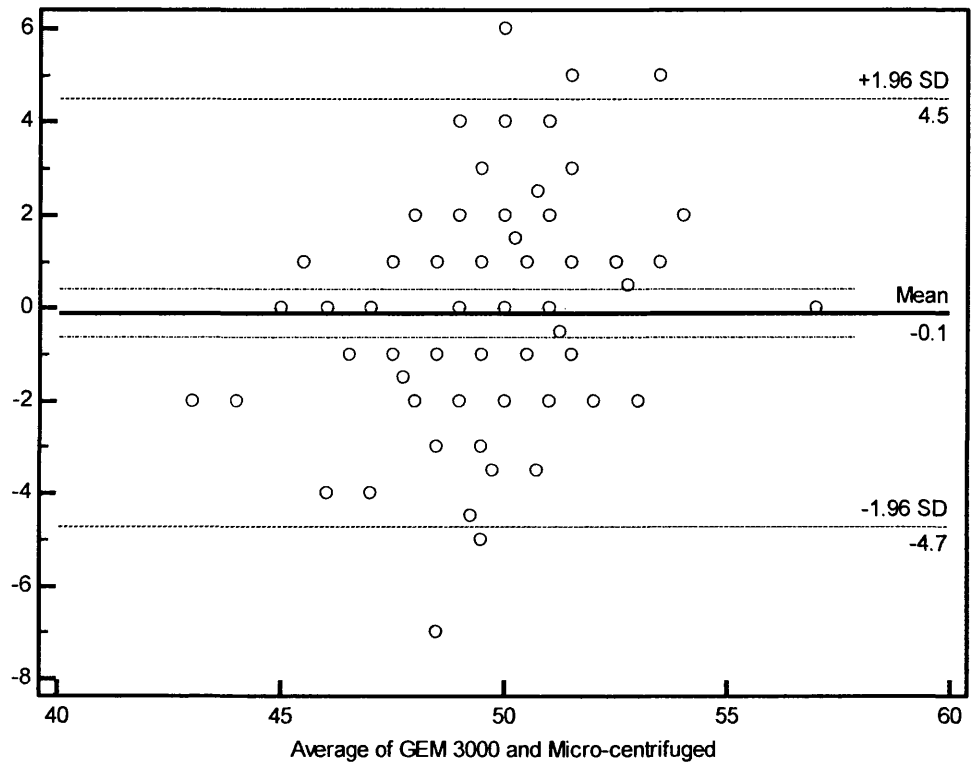


**Figure 7.1** Bland and Altman Plot

# Chapter 8

## References

## REFERENCES


Adopo, E., Peronnet, F., Massicotte, D., Brisson, G. R. and Hillaire-Marcel, C. (1994). Respective oxidation of exogenous glucose and fructose given in the same drink during exercise. *Journal of Applied Physiology*, 76, 1014-19.

Ali, A. and Williams, C. (2009). Carbohydrate ingestion and soccer skill performance during prolonged intermittent exercise. *Journal of Sports Sciences*, 27 (14), 1499-1508.

Ali, A., Williams, C., Hulse, M., Strudwick, A., Reddin, J., Howard, L. et al. (2007). Reliability and validity of two test of soccer skill. *Journal of Sports and Sciences, 25(13),* 1461-1470.

Armstrong, L. E. (2005). Hydration assessment techniques. *Nutrition Reviews*, 63 (6), S40-S54.

Armstrong, L. E., Casa, D. J., Maresh, C. M. and Ganio, M. S. (2007). Caffeine, fluid-electrolyte balance, temperature regulation, and exercise-heat tolerance. *Exercise and Sport Sciences Reviews*, 35 (3), 135-140.

Armstrong, L. E., Costill, D. L. and Fink, W. J. (1985). Influence of diuretic-induced dehydration on competitive running performance. *Medicine and Sciences in Sports and Exercise*, 17, 456-461.

Armstrong, L. E., Pumerantz, A. C., Fiala, K. A., Roti, M. W., Kavouras, S. A., Casa, D. J. and Maresh, C. M. (2010). Human hydration indices: acute and longitudinal reference values. *International Journal of Sport Nutrition and Exercise Metabolism*, 20 (2), 145-153.

Armstrong, L. E., Soto, J. A., Hacker, Jr. F. T., Casa, D. J., Kavouras, S. A. and Maresh, C. M. (1998). Urinary indices during dehydration, exercise, and rehydration. *International Journal of Sport Nutrition*, 8, 345-355.

Backhouse, S. H., Ali, A., Biddle, S. J. and Williams, C. (2007). Carbohydrate ingestion during prolonged high-intensity intermittent exercise: impact on affect and perceived exertion. *Scandinavian Journal of Medicine and Science in Sports*, 17 (5), 605-610.

Bangsbo, J. (1994). *The physiology of soccer.* Denmark: HO+Storm.

Bangsbo, J. (1995). *Fitness training in football: A scientific approach.* Bagsvard, Denmark: HO+Storm.

Bangsbo, J. and Krustrup, P. (2006). Physical and metabolic demands of training and match-play in the elite football player. *Journal of Sports Sciences*, 24 (7), 665-674.

Bangsbo, J., Iaia, F. M. and Krustrup, P. (2007). Metabolic response and fatigue in soccer. *International Journal of Sports Physiology and Performance*, 2, 111-127.

Bangsbo, J., Norregaard, L., and Thorso, F. (1991). Activity profile of professional soccer. *Canadian Journal of Sport Sciences,* 16 (2), 110-116.

Barros, R. M. L., Misuta, M. S., Menezes, R. P., Figueroa, P. J., Moura, F.A., Cunha, S. A., Anido, R. and Leite, N. J. (2007). Analysis of the distances covered by first division Brazilian soccer players obtained with an automatic tracking method. *Journal of Sports Science and Medicine*, 6, 233-242.

Benton, D. (2002). Carbohydrate ingestion, blood glucose and mood. *Neuroscience and Biobehavioral Reviews*, 26, 293-308.

Bishop, D. and Maxwell, N. S. (2009). Effects of active warm-up on thermoregulation and intermitten-sprint performance in hot conditions. *Journal of Science and Medicine in Sport*, 12, 196-204.

Bland, J. M. and Altman, D. G. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*, 1, 307-310.

Borg, G.A. (1973). Perceived exertion: a note on "history" and methods. *Medicine and Science in Sports, 5(2),* 90-93.

Bradley, P. S., Sheldon, W., Wooster, B., Olsen, P., Boanas, P. and Krustrup, P. (2009). High-intensity running in English FA League soccer matches. *Journal of Sports Sciences,* 27 (2), 159-168.

Brookes, J. D. and Knowles J. E. (1974). A movement analysis of player behaviour in soccer match performance. *Proceedings of the British Society of Sport Psychology Conference*, 246-256.

Brouns, F. and Beckers, E. (1993). Is the gut an athletic organ? *Digestion, absorption and exercise. Sports Medicine*, 15, 242-257.

Brouns, F., Senden, J., Beckers, E. J., Saris, W. H. (1995). Osmolality does not affect the gastric emptying rate of oral rehydration solutions. *Journal of Parenteral and Enteral Nutrition*, 19 (5), 403-406.

Campbell, C., Prince, D., Braun, M., Applegate, E. and Casazza, G. (2008). Carbohydrate-supplement form and exercise performance. *International Journal of Sport Nutrition and Exercise Metabolism*, 18 (2), 179-190.

Carling, C. and Bloomfield, J. (2010). The effect of an early dismissal on a player work-rate in a professional soccer match. *Journal of Science and Medicine in Sport*, 13 (1), 126-128.

Carling, C., Bloomfield, J., Nelsen, L. and Reilly, T. (2008). The role of motion analysis in elite soccer: contemporary performance measurement techniques and work rate data. *Sports Medicine*, 38 (10), 839-862.

Carling, C. and Dupont, G. (2011). Are declines in physical performance associated with a reduction in skill-related performance during professional soccer match-play? *Journal of Sports Sciences*, 29 (1), 63-71.

Casa, D. J., Amstrong, L. E., Hillman, S. K., Montain, S. J., Reiff, R. V., Rich, B. S. E., Roberts, W. O. and Stone, J. A. (2000). National Athletic Trainers' Association Position Statement: Fluid Replacement for Athletes. *Journal of Athletic Training*, 35 (2), 212-224.

Casa, D. J., Clarkson, P. M. and Roberts, W. M. (2005). American College of Sports Medicine roundtable on hydration and physical activity: consensus statement. *Current Sports Medicine Reports*, 4 (3), 115-127.

Cheuvront, S. N., Carter III, R., Castellani, J. W. and Sawka, M. N. (2005). Hypohydration impairs endurance exercise performance in temperate but not cold air. *Journal of Applied Physiology*, 99, 1972-1976.

Cheuvront, S. N., Carter III, R., Montain, S. J., Stephenson, L. A. and Sawka, M. N. (2004). Influence of hydration and air flow on thermoregulatory control in the heat. *Journal of Thermal Biology*, 29, 471-477.

Chryssanthopoulos, C.,Williams, C., Novitz, C., Kotsipoulou, C., and Vleck, V. (2002). The effect of a high carbohydrate meal on endurance running capacity. *International Journal of Sport Nutrition and Exercise Metabolism*, 12, 157-171.

Clarke, N. D., Drust, B., Maclaren, D. P. and Reilly, T. (2008). Fluid provision and metabolic responses to soccer-specific exercise. *European Journal of Applied Physiology*, 104 (6), 1069-1077.

Coombes, J. S. and Hamilton, K. L. (2000). The effectiveness of commercially available sport drinks. *Sports Medicine*, 29 (3), 181-209.

Costill, D. L. and Saltin, B. (1974). Factors limiting gastric emptying during rest and exercise. *Journal of Applied Physiology*, 37, 679-683.

Costill, D. L., Dalsky, G.P. and Fink, W. J. (1978). Effects of caffeine ingestion on metabolism and exercise performance. *Medicine and Science in Sports*, 10, 155-158.

Cox, G. R., Desbrow, B., Montgomery, P. G., Anderson, M. E., Bruce, C. R., Macrides, T. A., Martin, D. T., Moquin, A., Roberts, A., Hawley, J. A. and Burke, L. M. (2002). Effect of different protocols of caffeine intake on metabolism and endurance performance. *Journal of Applied Physiology*, 93, 990-999.

Coyle, E. F., Coggan, A. R., Hemmert, M. K. and Ivy, J. L. (1986). Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *Journal of Applied Physiology,* 61, 165-172.

Currell, K., Conway, S. and Jeukendrup, A. E. (2009). Carbohydrate ingestion improves performance of a new reliable test of soccer performance. *International Journal of Sport Nutrition and Exercise Metabolism*, 19, 34-46.

Davis, J. M., Burgess, W. A., Slentz, C. A. and Bartoli, W. P. (1990). Fluid availability of sports drinks differing in carbohydrate type and concentration. *American Journal of Clinical Nutrition*, 51 (6), 1054-1057.

Del Coso, J., Estevez, E. and Mora-Ridriguez, R. (2009). Caffeine during exercise in the heat: thermoregulation and fluid-electrolyte balance. *Medicine and Sciences in Sports and Exercise*, 41 (1), 164-173.

Desbrow, B., Barret, C. M., Minahan, C. L., Grant, G. D. and Leveritt, M. D. (2009). Caffeine, cycling performance, and exogenous CHO oxidation: a dose-response study. *Medicine and Sciences in Sports and Exercise*, 41 (9), 1744-1751.

Di Salvo, V., Gregson, W., Atkinson, G., Tordoff, P. and Drust, B. (2009). Analysis of high intensity activity in Premier League soccer. *International Journal of Sports Medicine*, 30 (3), 205-212.

Dill, D.B. and Costill, D.L. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *Journal of Applied Physiology,* 37 (2), 247-248.

Drust, B., Reilly, T. and Cable, N. T. (2000). Physiological response to laboratory-based soccer-specific intermittent and continuous exercise. *Journal of Sports Medicine*, 18, 885-892.

Duchman, S. M., Bleiler, T. L., Schedl, H. P., Summers, R. W., Bleiler, T. L. and Gisolfi, C. V. (1997). Upper limit for intestinal absorption of a dilute glucose solution in men at rest. *Medicine and Science in Sports and Exercise*, 29 (4), 482-488.

Dvorak, J. (2011). Osteoarthritis in football: FIFA/F-MARC approach. *British Journal of Sports Medicine*, 45, 673-676.

Edwards, A. M., Mann, M. E., Marfell-Jones, M. J., Rankin, D. M., Noakes, T. D. and Shillington, D. P. (2007). Influence of moderate dehydration on soccer performance: physiological responses to 45 min of outdoor match-play and the immediate subsequent performance of sport-specific and mental concentration tests. *British Journal of Sports Medicine*, 41 (6), 385-391.

Ekblon, B. (1986). Applied physiology of soccer. *Sports Medicine*, 3 (l), 50-60.

Evans, G. H., Shirreffs, S. M. and Maughan, R. J. (2009). Acute effects of ingesting glucose solutions on blood and plasma volume. *British Journal of Nutrition*, 101 (10), 1503-08.

Fallon, K. D., Ehrmeyer, S. S., Laessig, R. H., Mansouri, S. and Ancy, J. J. (2003). From quality control and quality assurance to assured quality. *Point of Care: The Journal of Near-Patient Testing & Technology*, 2 (3), 188-194.

Ferraris, R. P. (2001). Dietary and developmental regulation of intestinal sugar transport. *Biochemical Journal*, 360, 265-276.

FIFA (2006). Nutrition for football: The FIFA/F-MARC Consensus Conference. *Journal of Sports Sciences*, 24 (7), 663-664.

Fink, W. L., Costill, D. L., Van Handel, P.J. (1975). Leg muscle metabolism during exercise in the heat and the cold. *European Journal of Applied Physiology*, 34, 183-190.

Fitts, R. H. (1994). Cellular mechanisms of muscle fatigue. *Physiological Review*, 74, 49-94.

Fitts, R. H. and Balog, E. M. (1996). Effects of intracellular and intracellular ion changes on E-C coupling and skeletal muscle fatigue. *Acta physiologica Scandinavica*, 156, 169-181.

Flynn, M. G., Costill, D. L., Hawley, J. A., Fink, W. J., Neufer, P. D., Fielding, R. A., Sleeper, M. D. (1987). Influence of selected carbohydrate drinks on cycling performance and glycogen use. *Medicine and Science in Sports and Exercise*, 19 (1), 37-40.

Foskett, A., Williams, C., Boobis, L. and Tsintzas, K. (2008). Carbohydrate availability and muscle energy metabolism during intermittent running. *Medicine and Science in Sports and Exercise*, 40 (1), 96-103.

Fredholm, B. B., Battig, K., Holmen, J., Nehlig, A. and Zvartau, E. E. (1999). Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacological Reviews*, 51, 83-133.

Frier, B. (2002). Epidemiology, short and long-term consequences of hypoglycaemia. *Diabetes, Nutrition and Metabolism*, 15, 378-385.

Gaitanos, G.C., Williams, C., Boobis, L.H. and Brooks, S. (1993) Human muscle metabolism during intermittent maximal exercise. *Journal of Applied Physiology, 75,* 712-719.

Galloway, S. D. R., Wootton, S. A., Murphy, J. L. and Maughan, R. J. (2001). Exogenous carbohydrate oxidation from drinks ingested during prolonged exercise in a cold environment in humans. *Journal of Applied Physiology*, 91, 654-660.

Gant, N., Ali, A. and Foskett, A. (2010). The influence of caffeine and carbohydrate coingestion on simulated soccer performance. *International Journal of Sport Nutrition and Exercise Metabolism*, 20 (3), 191-197.

Gisolfi, C. V. (2000). Is the GI system built for exercise? *News in Physiological Sciences*, 15, 114-119.

Gisolfi, C. V., Summers, R. W., Lambert, G. P. and Xia, T. (1998). Effect of beverage osmolality on intestinal fluid absorption during exercise. *Journal of Applied Physiology*, 85 (5), 1941-48.

Gisolfi, C. V., Summers, R. W., Schedl, H. P. and Bleiler, T. L. (1992). Intestinal water absorption from select carbohydrate solutions in humans. *Journal of Applied Physiology*, 73 (5), 2142-50.

Glaister, M., Howatson, G., Abraham, C. S., Lockey, R. A., Goodwin, J. E., Foley, P. and McInnes, G. (2008). Caffeine supplementation and multiple sprint running performance. *Medicine and Science in Sports and Exercise*, 40 (10), 1835-1840.

Gonzalez-Alonso, J., Calbet, J. A. and Nielsen, B. (1998). Muscle blood flow is reduced with dehydration during exercise in humans. *Journal of Physiology*, 513 (3), 895-905.

Gonzalez-Alonso, J., Teller, C., Andersen, S. L., Jensen, F. B., Hyldig, T. and Nielsen, B. (1999). Influence of body temperature on the development of fatigue during prolonged exercise in the heat. *Journal of Applied Physiology*, 86 (3), 1032-1039.

Graham, T. E. and Spriet, L. L. (1991). Performance and metabolic responses to a high caffeine dose during prolonged exercise. *Journal of Applied Physiology*, 71 (6), 2292-2298.

Hamilton, P. and Bickle, I. (2006). *Data Interpretation for Medical Students.* Knutsford, UK; PasTest Ltd.

Hargreaves, M., Dillo, P., Angus, D. and Febbraio, M. (1996). Effect of fluid ingestion on muscle metabolism during prolonged exercise. *Journal of Applied Physiology*, 80, 363-366.

Harland, B. (2000). Caffeine and nutrition. *Nutrition*, 16, 522-526.

Harrison, M. H. (1985). Effects of thermal stress and exercise on blood volume in humans. *Physiological Review,* 65, 149-209.

Hughes, M. and Franks, I. (2005). Analysis of passing sequences, shots and goals in soccer. *Journal of Sports Sciences*, 23, 509-514.

Hulston, C. J., Wallis, G. A. and Jeukendrup, A. E. (2009). Exogenous CHO oxidation with glucose plus fructose intake during exercise. *Medicine and Science in Sports and Exercise*, 41 (2), 357-363.

Hunt, J. B., Elliot, E. J., Fairclough, P. D., Clark, M. L. and Farthing, M. J. (1992). Water and solute absorption from hypotonic glucose-electrolyte solution in human jejunum. *Gut*, 33 (4), 479-483.

Hunter, A. M., Gibson, A. S., Collins, M., Lambert, M. and Noakes, T. D. (2002). Caffeine ingestion does not alter performance during a 100-km cycling time-trial performance. *International Journal of Sport Nutrition and Exercise Metabolism*, 12, 438-452.

Ivy, J. L., Costill, D. L., Fink, W. J. and Lower, R. W. (1979). Influence of caffeine and carbohydrate feeding on endurance performance. *Medicine and Science in Sports*, 11 (1), 6-11.

Jacobs, I., Westlin, N., Karlson, J., Rasmusson, M. and Houghton, B. (1982). Muscle glycogen and diet in elite soccer players. *European Journal of Applied Physiology*, 48, 297-302.

Jacobson, B. H. and Kulling, F. A. (1989). Health and ergogenic effects of caffeine. *British Journal of Sports Medicine*, 23 (1), 34-40.

Jacobson, T. L., Febbraio, M. A., Arkinstall, M. J., Hawley, J. A. (2001). Effect of caffeine coingested with carbohydrate or fat on metabolism and performance in endurance-trained men. *Experimental Physiology*, 86, 137-144.

Jandrain, B. J., Pallikaris, N., Normand, S., Pirnay, F., Lacroix, M., Mosora, F., Pachiaudi, C., Gautier, J. F., Scheen, A. J., Riou, J. P. and Lefebvre, P. J. (1993). Fructose utilization during exercise in men: rapid conversion of ingested fructose to circulating glucose. *Journal of Applied Physiology*, 74, 2146-54.

Jellinger, P. (2007). Metabolic consequences of hyperglycemia and insulin resistance. *Clinical Cornerstone*, 8, S30-42.

Jentjens, R. L. and Jeukendrup, A. E. (2005a). High rates of exogenous carbohydrate oxidation from a mixture of glucose and fructose ingested during prolonged cycling exercise. *British Journal of Nutrition*, 93(4), 485-492.

Jentjens, R. L., Moseley, L., Waring, R. H., Harding, L. K. and Jeukendrup, A. E. (2004). Oxidation of combined ingestion of glucose and fructose during exercise. *Journal of Applied Physiology*, 96, 1277-1284.

Jentjens, R. L., Shaw, C., Birtles, T., Waring, R. H., Harding, L. K. and Jeukendrup, A. E. (2005b). Oxidation of combined ingestion of glucose and sucrose during exercise. *Metabolism Clinical and Experimental*, 54, 610-618.

Jentjens, R. L., Underwood, K., Achten, J., Currell, K., Mann, C. H. and Jeukendrup, A. E. (2006). Exogenous carbohydrate oxidation rates are elevated after combined ingestion of glucose and fructose during exercise in the heat. *Journal of Applied Physiology*, 100 (3), 807-816.

Jeukendrup, A. E. and Jentjens, R. (2000). Oxidation of carbohydrate feedings during prolonged exercise: current thoughts, guidelines and directions for future research. *Sports Medicine*, 29 (6), 407-424.

Jeukendrup, A. E., Raben, A., Gijsen, A., Stegen, J., Brouns, F., Saris, W. and Wagenmakers, A. (1999). Glucose kinetics during prolonged exercise in highly trained human subjects: effect of glucose ingestion. *The Journal of Physiology*, 515 (2), 579-589.

Johansen, L. B., Videbaek, R., Hammerum, M. and Norsk, P. (1998). Understimation of plasma volume changes in humans by hematocrit/haemoglobin method. *American Journal of Physiology*, 274, R126-130.

Karelis, A. D., Smith, J. W., Passe, D. H. and Peronnet, F. (2010). Carbohydrate administration and exercise performance: What are the potential mechanisms involved? *Sports Medicine*, 40, 747-763.

Kavouras, S. (2002). Assessing hydration status. *Current Opinion in Clinical Nutrition and Metabolic Care*, 5, 519-524.

Kingsley, M. I., Wadsworth, D., Kilduff, L. P., McEneny, J. and Benton, D. (2005). Effects of phosphatidylserine on oxidative stress following intermittent running. *Medicine and Science in Sports and* Exercise, 37, 1300-1306.

Kovacs, E. M, Senden, J. M. and Brouns, F. (1999). Urine colour, osmolality, and specific electrical conductance are not accurate measures of hydration status during post-exercise rehydration. *Journal of Sports Medicine and Physical Fitness*, 39, 47-53.

Kovacs, E. M., Stegen, J. H. C. H. and Brouns, F. (1998). Effect of caffeinated drinks on substrate metabolism, caffeine excretion, and performance. *Journal of Applied Physiology*, 85, 709-715.

Krustrup, P., Mohr, M., Ellingsgaard, H. and Bangsbo, J. (2005). Physical demands of elite female soccer games: Importance of training status. *Medicine and Science in Sports and Exercise*, 37, 1242-1248.

Krustrup, P., Mohr, M., Steensberg, A., Bencke, J., Kjar, M. and Bangsbo, J. (2003). Muscle metabolites during a football match in relation to a decreased sprinting ability. In *Part III: Physiology and Kinanthropometry*. London: Taylor & Francis.

Krustrup, P., Mohr, M., Steensberg, A., Bencke, J., Kjær, M. and Bangsbo, J. (2006). Muscle and blood metabolites during a soccer game: Implications for sprint performance. *Medicine and Science in Sports and Exercise, 38(6)*, 1165-1174.

Kuipers, H., Fransen, E. J. and Keizer, H.A. (1999). Pre-exercise ingestion of carbohydrate and transient hypoglycemia during exercise. *International Journal of Sports Medicine*, 20, 227-231.

Kurdak, S. S., Shirreffs, S .M., Maughan, R. J., Ozgünen, K. T., Zeren, C., Korkmaz, S., Yazidi, Z., Ersöz, G., Binnet, M. S. and Dvorak, J. (2010). Hydration and sweating responses to hot-weather football competition. *Scandinavian Journal of Medicine and Science in Sports*, 20 (3), 133-139.

Lago, C., Casais, L., Dominguez, E. and Sampaio, J. (2010). The effects of situational variables on distance covered at various speeds in elite soccer. *European Journal of Sport Science*, 10, 103-109.

Lamb, G. D. and Stephenson, D. G. (1994). Effects of intracellular pH and [Mg2+] on excitation-contraction coupling in skeletal muscle fibres of the rat. *Journal of Physiology*, 478, 331-339.

Lambert, P., Lanspa, S., Welch, R. and Shi, X. (2008). Combined effects of glucose and fructose on fluid absorption from hypertonic carbohydrate-electrolyte beverages. *Journal of Exercise Physiology Online*, 11 (2), 46-55.

Lambert, E. V., St Clair-Gibson, A. and Noakes, T. D. (2005). Complex systems model of fatigue: integrative homeostatic control of peripheral systems during exercise in humans. *British Journal of Sports Medicine*, 39, 52-62.

Leatt, P. B. and Jacobs, I. (1989). Effect of glucose polymer ingestion on glycogen depletion during a soccer match. *Canadian Journal of Sport Sciences*, 14 (2), 112-116.

Leiper, J. B. and Maughan, R. J. (1986). Absorption of water and electrolytes from hypotonic, isotonic and hypertonic solutions. *The Journal of Physiology, 373,* 90P.

Leiper, J. B., Nicholas, C. W., Ali, A., Williams, C. and Maughan, R. J. (2005). The effect of intermittent high-intensity running on gastric emptying of fluids in man. *Medicine and Science in Sport and Exercise*, 37 (2), 240-247.

Leiper, J. B., Broad, N. P. and Maughan, R. J. (2001a). Effect of intermittent high-intensity exercise on gastric emptying in man. *Medicine and Science in Sports and Exercise*, 33 (8), 1270-1278.

Leiper, J. B., Prentice, A. S., Wrightson, C. and Maughan, R. J. (2001b). Gastric emptying of a carbohydrate-electrolyte drink during a soccer match. *Medicine and Science in Sports and Exerc*ise, 33, 1932-38.

Linnane, D. M., Bracken, R. M., Brooks, S., Cox, V. M. and Ball, D. (2004). Effects of hyperthermia on the metabolic responses to repeated high-intensity exercise. *European Journal of Applied Physiology*, 93 (1-2), 159-166.

Lionne, C., Brune, M., Webb, M. R., Travers, F. and Barman, T. (1995). Time resolved measurements show that phosphate release is the rate limiting step on myofibrillar ATPases. *FEBS Letters*, 364, 59-62.

Lugo, M., Sherman, W. M., Wimer, G. S. and Garleb, K. (1993). Metabolic responses when different forms of carbohydrate energy are consumed during cycling. *International Journal of Sport Nutrition*, 3 (4), 398-407.

Lyons, M., Al-Nakeeb, Y. and Nevill, A. (2006). Performance of soccer passing skills under moderate and high-intensity localized muscle farigue. *Journal of Strength and Conditioning Research*, 20 (1), 197-202.

Mainwood, G. W., Renaud, J. M. and Mason, M. J. (1987). The pH dependence of the contractile response of fatigue skeletal muscle. *Canadian Journal of Physiology and Pharmacology*, 65 (4), 648-658.

Marmy-Conus, N., Fabris, S., Proietto, J. and Hargreaves, M. (1996). Preexercise glucose ingestion and glucose kinetics during exercise. *Journal of Applied Physiology*, 81 (2), 853-857.

Massicotte, D., Péronnet, F., Adopo, E., Brisson, G. R., Hillaire-Marcel, C. (1994). Effect of metabolic rate on the oxidation of ingested glucose and fructose during exercise. *International Journal of Sports Medicine*, 15 (4), 177-180.

Massicotte, D., Peronnet, F., Brisson, G., Bakkouch, K. and Hillaire-Marcel, C. (1989). Oxidation of a glucose polymer during exercise: comparison with glucose and fructose. *Journal of Applied Physiology*, 66, 179-183.

Maughan, R. J., Fenn, C. E., Leiper, J. B. (1989). Effects of fluid, electrolyte and substrate ingestion on endurance capacity. *European Journal of Applied Physiology*, 58, 481-486.

Maughan, R. J. and Leiper, J. B. (1994). Fluid replacement requirements in soccer. *Journal of Sports Sciences*, 12, S29-34.

Maughan, R. J., Merson, S. J., Broad, N. P. and Shirreffs, S. M. (2004). Fluid and electrolyte intake and loss in elite soccer players during training. *International Journal of Sport Nutrition and Exercise Metabolism*, 14 (3), 333-346.

Maughan, R. J. and Shirreffs, S. M. (2010). Dehydration and rehydration in competitive sport. *Scandinavian Journal of Medicine and Science in Sports*, 20 (3), 40-47.

Maughan, R. J., Shirreff, S. M., Merson, S. J. and Horswill, C. A. (2005). Fluid and electrolyte balance in male football (soccer) players training in a cool environment. *Journal of Sports Sciences*, 23, 73-79.

Maughan, R. J., Shirreffs, S. M. and Leiper, J. B. (2007a). Errors in the estimation of hydration status from changes in body mass. *Journal of Sports Sciences,* 25 (7), 797-804.

Maughan, R. J., Watson, P., Evans, G. H., Broad, N. and Shirreffs, S. M. (2007b). Water balance and salt losses in competitive football. *International Journal of Sports Nutrition and Exercise Metabolism*, 17, 583-594.

McArdle, W. D., Katch, F. I. and Katch, V. L. (2006). *Exercise physiology: Energy, nutrition, and human performance* (6th ed.). Baltimore: Williams & Wilkins.

McGregor, S. J., Nicholas, C. W., Lakomy, H. K. and Williams, C. (1999). The influence of intermittent high-intensity shuttle running and fluid ingestion on the performance of a soccer skill. *Journal of Sports Sciences, 17,* 895-903.

Meeussen, R., Watson, P. and Dvorak, J. (2006). The brain and fatigue: new opportunities for nutritional interventions. *Journal of Sports Sciences*, 24, 773-782.

Millard-Stafford, M. L., Cureton, K. J., Wingo, J. E., Trilk, J., Warren, G. L. and Buyckx, M. (2007). Hydration during exercise in warm, humid conditions: effect of a caffeinated sport drink. *International Journal of Sport Nutrition and Exercise Metabolism*, 17 (2), 163-177.

Millard-Stafford, M., Sparling, P., Rosskopf, L. and DiCarlo, L. (1992). Carbohydrate-electrolyte replacement improves distance running in the heat. *Medicine and Science in Sports and Exercise*, 24 (8), 934-940.

Mitchell, J. B., Costill, D. L., Houmard, J. A., Fink, W. J., Pascoe, D. D. and Pearson, D. R. (1989). Influence of carbohydrate dosage on exercise performance and glycogen use. *Journal of Applied Physiology*, 67, 1843-1849.

Mitchell, J. W., Nadel, E. R. and Stolwijk, J. A. (1972). Respiratory weight losses during exercise. *Journal of Applied Physiology*, 32 (4), 474-476.

Morh, M., Krustup, P. and Bangsbo, J. (2003). Match performance of high-standard soccer players with special reference to development of fatigue. *Journal of Sports Sciences, 21(7),* 519-28.

Mohr, M., Krustrup, P. and Bangsbo, J. (2005). Fatigue in soccer: A brief review. *Journal of Sports Sciences*, 23, 593-599.

Mohr, M., Krustrup, P., Nybo, L., Nielsen, J. J. and Bangsbo, J. (2004a). Muscle temperature and sprint performance during soccer matches - beneficial effects of re-warm-up at half time. *Scandinavian Journal of Medicine and Science in Sports*, 14, 156-162.

Mohr, M., Mujika, I., Santisteban, J., Randers, M. B., Bischoff, R., Solano, R., Hewitt, A., Zubillaga, A., Peltola, E. and Krustrup, P. (2010). Examination of fatigue development in elite soccer in a hot environment: a multi-experimental approach. *Scandinavian Journal of Medicine and Science in Sports*, 20 (3), 125-132.

Mohr, M., Nordsborg, N., Nielsen, J. J., Pedersen, L. D., Fisher, C., Krustrup, P., Bangsbo, J. (2004b). Potassium kinetics in human muscle interstitium during repeated intense exercise in relation to fatigue. *Plügers Archiv - European Journal of Physiology*, 448 (4), 452-456.

Montain, S. J., Cheuvront, S. N. and Sawka, M. N. (2006). Exercise associated hyponatraemia: quantitative analysis to understand the aetiology. *British Journal of Sports Medicine*, 40 (2), 98-105.

Montain, S. J. and Coyle, E. F. (1992). Influence of dehydration and hyperthermia on cardiovascular drift during exercise. *Journal of Applied Physiology*, 73, 1340-1350.

Morris, J. G., Nevill, M. E., Boobis, L. H., Macdonald, I. A. and Williams, C. (2005). Muscle metabolism, temperature, and function during prolonged, intermittent, high-intensity running in air temperature of 33 degrees and 17 degrees C. *International Journal of Sports Medicine*, 26 (10), 805-814.

Morris, J. G., Nevill, M. E., Thompson, D., Collie, J. and Williams, C. (2003). The influence of a 6.5% carbohydrate-electrolyte solution on performance of prolonged intermittent high-intensity running at 30 degrees C. *Journal of Sports Sciences*, 24 (5), 371-381.

Motl, R. W., O'Connor, P. J., Tubandt, L., Puetz, T. and Ely, M.R. (2006). Effect of caffeine on leg pain during cycling exercise among females. *Medicine and Science in Sports and Exercise*, 38 (3), 598-604.

Murdoch, S. D., Bazzarre, T.L., Snider, I. P. and Goldfarb, A. H. (1993). Differences in the effects of carbohydrate food form on endurance performance to exhaustion. *International Journal of Sport Nutrition*, 3 (1), 41-54.

Murray, R., Seifert, J. G., Eddy, D. E., Paul, G. L. and Halaby, G. A. (1989). Carbohydrate feeding and exercise: effect of beverage carbohydrate content. *European Journal of Applied Physiology and Occupational Physiology*, 59 (1-2), 152-158.

Mustafa, K. Y. and Mahmoud, N. E. (1979). Evaporative water loss in African soccer players. *Journal of Sports Medicine and Physical Fitness*, 19 (2), 181-183.

Neuhauser-Berthold, B. S., Verwied, S. C. and Luhrmann, P. M. (1997). Coffee consumption and total body water homeostasis as measured by fluid balance and bioelectrical impedance analysis. *Annals of Nutrition and Metabolism*, 41, 29-36.

Newsholme, E. A., Ackworth, I. and Blomstrand, E. (1987). Amino acids, brain neurotransmitters and a function link between muscle and brain that is important in sustained exercise. In G. Benzi (Ed.), *Advances in myochemistry* (pp. 127-133). London, UK: John Libbey Eurotext.

Nicholas, C. W., Nuttall, F. E. and Williams, C. (2000). The Loughborough Intermittent Shuttle Test: A field test that simulates the activity pattern of soccer. *Journal of Sports Sciences, 18,* 97-104.

Nicholas, C. W., Tsintzas, K., Boobis, L. and Williams, C. (1999). Carbohydrate-electrolyte ingestion during intermittent high-intensity running. *Medicine and Science in Sports and Exercise*, 31 (9), 1280-1286.

Nicholas, C. W., Williams, C., Lakomy, H. K., Phillips, G. and Nowitz, A. (1995). Influence of ingesting a carbohydrate-electrolytes solution on endurance capacity during intermittent, high-intensity shuttle running. *Journal of Sports Sciences*, 13, 283-290.

Nielsen, J. J., Mohr, M., Klarskov, C., Kristensen, M., Krustrup, P., Juel, C. and Bangsbo, J. (2004). Effects of high-intensity intermittent training on potassium kinetics and performance in human skeletal muscle. *Journal of Physiology*, 554, 857-870.

Niswender, K. and Beech, B. (2008). Obesity: increasing awareness of novel environmental factors. *Diabetes*, 57, 1786-1787.

Nybo, L. (2003). CNS fatigue and prolonged exercise: effect of glucose supplementation. *Medicine and Science in Sports and Exercise*, 34 (4), 589-594.

Nybo, L. and Secher, N.H. (2004). Cerebral perturbations provoked by prolonged exercise. *Progress in Neurobiology*, 72, 223-261.

Ohashi, J., Togari, H., Isokawa, M. and Suzuki, S. (1988). Measuring movement speeds and distances covered during soccer match-play. In: Reilly T, Lees A, Davids K, Murphy WJ, eds. Science and football. London: E&FN Spon.

Oppliger, R. A. and Bartok, C. (2002). Hydration testing of athletes. *Sports Medicine*, 32 (15), 959-971.

Ostojic, S. M. and Mazic, S. (2002). Effects of a carbohydrate-electrolyte drink on specific soccer test and performance. *Journal of Sports Sciences and Medicine*, 1, 47-53.

Ozgünen, K. T., Kurdak, S. S., Maughan, R. J., Zeren, C., Korkmaz, S., Yazici, Z., Ersöz, G., Shirreffs, S. M., Binnet, M. S. and Dvorak, J. (2010). Effect of hot environmental conditions on physical activity patterns and temperature response of football players. *Scandinavian Journal of Medicine and Science in Sports*, 20 (3), 140-147.

Pasman, W. J., van Baak, M. A., Jeukendrup, A. E. and de Haan, A. (1995). The effect of different dosages of caffeine on endurance performance time. *International Journal of Sports Medicine*, 16, 225-230.

Passmore, A. P., Kondowe, G. P. and Johnston, G. D. (1987). Renal and cardiovascular effects of caffeine: a doseresponse study. *Clinical Science*, 72, 749-756.

Patterson, S. D. and Gray, S. C. (2007). Carbohydrate-gel supplementation and endurance performance during intermittent high-intensity shuttle running. *International Journal of Sport Nutrition and Exercise Metabolism*, 17, 445-455.

Pfeiffer, B., Cotterill, A., Grathwohl, D., Stellingwerff, T. and Jeukendrup, A. E. (2009). The effect of carbohydrate gels on gastrointestinal tolerance during a 16-km run. *International Journal of Sports Nutrition and Exercise Metabolism*, 19 (5), 485-503.

Pfeiffer, B., Stellingwerff, T., Hodgson, A. B., Randell, R., Pottgen, K, Res, P. and Jeukendrup, A. E. (2011). Nutritional intake and gastrointestinal problems during competitive endurance events. *Medicine and Science in Sports and Exercise*, 19 [Epub ahead of print].

Pfeiffer, B., Stellingwerff, T., Zaltas, E. and Jeukendrup, A. E. (2010). CHO oxidation from a CHO gel compared with a drink during exercise. *Medicine and Science in Sports and Exercise*, 42 (11), 2038-45.

Phillips, S. M., Turner, A. P., Gray, S., Sanderson, M. F. and Sproule, J. (2010). Ingesting a 6% carbohydrate-electrolyte solution improves endurance capacity, but not sprint performance, during intermittent, high-intensity shuttle running in adolescent team games players aged 12-14 years. *European Journal of Applied Physiology*, 109 (5), 811-821.

Phillips, S. M., Turner, A. P., Sanderson, M. F. and Sporule, J. (2011). Carbohydrate gel ingestion significantly improves the intermittent endurance capacity, but not sprint performance, of adolescent team games players during a simulated team games protocol. *European Journal of Applied Physiology*, 13, [Epub ahead of print].

Popowski, L. A., Oppliger, R. A., Patrick, L. G., Johnson, R. F., Kim, J. A. and Gisolfi, C. V. (2001). Blood and urinary measures of hydration status during progressive acute dehydration. *Medicine and Science in Sports and Exercise*, 33 (5), 747-753.

Rampinini, E., Coutts, A. J., Castagna, C., Sassi, R. and Impellizzeri, F. M. (2007). Variation in Top Level Soccer Match Performance. *International Journal of Sports Medicine*, 28, 1018-1024.

Rampinini, E., Impellizzeri, F. M., Castagna, C., Coutts, A. J., Wisløff, U. (2009). Technical performance during soccer matches of the Italian Serie A league: effect of fatigue and competitive level. *Journal of Science and Medicine in Sport*, 12 (1), 227-233.

Ramsbottom, R., Brewer, J. and Williams, C. (1988). A progressive shuttle run test to estimate maximal oxygen uptake. *British Journal of Sport Medicine, 22 (4)*, 141-144.

Rehrer, N. J., Beckers, E. J., Brouns, F., Saris, W. H. and Ten Hoor, F. (1993). Effects of electrolytes in carbohydrates beverages on gastric emptying and secretion. *Medicine and Science in Sports and Exercise*, 25 (1), 42-51.

Rehrer, N. J., Brouns, F., Beckers, E. J., Ten Hoor, F. and Saris, W. H. (1990). Gastric emptying with repeated drinking during running and bicycling. *International Journal of Sports Medicine*, 11 (3), 238-243.

Rehrer, N. J.,Wagenmakers, A. J. M., Beckers, E. J., Halliday, D., Leiper, J. B., Brouns, F., Maughan, R. J., Westerperp, K. and Saris, W. H. (1992). Gastric emptying, absorption and carbohydrate oxidation during prolonged exercise. *Journal of Applied Physiology*, 72 (2), 468-475.

Reilly, T. (1997). Energetics of high-intensity exercise (soccer) with particular reference to fatigue. *Journal of Sports Sciences*, 15 (3), 257-263.

Reilly, T. and Ekblom, B. (2005). The use of recovery methods postexercise. *Journal of Sports* Sciences, 23, 619-627.

Reilly, T. and Thomas, V. (1976). A motion analysis of work-rate in different positional roles in professional football match play. *Journal of Human Movement Studies*, 7, 87-97.

Rienzi, E., Drust, B., Reilly, T., Carter, J. E. L., Martin, A. (2000). Investigation of anthropometric and work-rate profiles of elite South American international soccer players. *Journal of Sports Medicine and Physical Fitness*, 40 (2), 162-169.

Rodriguez, N. R., DiMarco, N. M., Langley, S., American Dietetic Association, Dietitians of Canada, American College of Sports Medicine (2009). Join position statement: Nutrition and athletic performance. *Journal of the American Dietician Association*, 109 (3), 509-527.

Romijn, J. A., Coyle, E. F., Sidossis, L. S., Gastaldelli, A., Horowitz, J. F., Endert, E. and Wolfe, R. R. (1993). Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *American Journal of Physiology*, 265, E380-391.

Rosner, M. H. (2008). Exercise-associated hyponatremia. *The Physician and Sports Medicine*, 36 (1), 55-61.

Rowell, L. B. (1974). Human cardiovascular adjustments to exercise and thermal stress. *Physiological Review*, 54, 75-159.

Rowlands, D. S., Thorburn, M. S., Thorp, R. M., Broadbent, S., Shi, X. (2008). Effect of graded fructose coingestion with maltodextrin on exogenous 14C-fructose and 13C-glucose oxidation efficiency and high-intensity cycling performance. *Journal of Applied Physiology*, 104, 1709-1719.

Russell, M., Benton, D. and Kingsley, M. (2010). Reliability and construct validity of soccer skills tests that measure passing, shooting, and dribbling. *Journal of Sports Sciences*, 28 (13), 1399-1408.

Russell, M., Benton, D. and Kingsley, M. (2011b). The effects of fatigue on soccer skills performed during a soccer match simulation. *International Journal of Sports Physiology and Performance*, 6 (2), 221-233.

Russell, M., Rees, G., Benton, D. and Kingsley, M. (2011a). An exercise protocol that replicates soccer match-play. *International Journal of Sports Medicine*, 32 (7), 511-518.

Saltin, B. (1973). Metabolic fundamentals in exercise. *Medicine and Science in Sports, 5*, 137-46.

Sawka, M. N., Burke, L. M., Eichner, E. R., Maughan, R. J., Montain, S. J. and Stachenfeld, N. S. (2007). American College of Sports Medicine position stand. Exercise and fluid replacement. *Medicine and Science in Sports and Exercise*, 39 (2), 377-390.

Shephard, R. J. (1992). The energy needs of the soccer player. *Clinical Journal of Sports Medicine*, 2, 62-70.

Shephard, R. J. (1999). Biology and medicine of soccer: An update. *Journal of Sports Sciences*, 17, 757-786.

Shephard, R. J. and Leatt, P. (1987). Carbohydrate and fluid needs of the soccer player. *Sports Medicine*, 4, 164-176.

Sherman, W. M., Peden, M. C. and Wright, D. A. (1991). Carbohydrate feedings 1 h before exercise improves cycling performance. *American Journal of Clinical Nutrition,* 54, 866-870.

Shi, X. and Gisolfi, C. V. (1998). Fluid and carbohydrate replacement during intermittent exercise. *Sports Medicine*, 25 (3), 157-172.

Shi, X. and Passe, D. H. (2010). Water and solute absorption from carbohydrate-electrolyte solutions in the human proximal small intestine: a review and statistical analysis. *International Journal of Sport Nutrition and Exercise Metabolism*, 20, 427-442.

Shi, X., Summers, R. W., Schedl, H. P., Chang, R. T., Lambert, G. P., Gisolfi, C. V. (1994). Effects of solution osmolality on absorption of select fluid replacement solutions in human duodenojejunum. *Journal of Applied Physiology*, 77 (3), 1178-84.

Shi, X., Summers, R. W., Schedl, H. P., Flanagan, S. W., Chang, R. and Gisolfi, C. V. (1995). Effects of carbohydrate type and concentration and solution osmolality on water absorption. *Medicine and Science in Sports and Exercise*, 27 (12), 1607-15.

Shirreffs, S. M. (2003). Markers of hydration status. *European Journal of Clinical Nutrition*, 57 (2), 6-9.

Shirreffs, S. M. (2010). Hydration: special issues for playing football in warm and hot environments. *Scandinavian Journal of Medicine and Science in Sports*, 20 (3), 90-94.

Shirreffs, S. M. and Maughan, R. J. (1998). Urine osmolality and conductivity as indices of hydration status in athletes in the heat. *Medicine and Science in Sports and Exerc*ise, 30 (11), 1598-1602.

Shirreffs, S. M., Ragon-Vargas, L. F., Chamorro, M., Maughan, R. J., Serratosa, L. and Zachwieja, J. J. (2005). The sweating response of elite professional soccer players to training in the heat. *International Journal of Sports Medicine*, 26, 90-95.

Shirreffs, S. M., Sawka, M. N. and Stone, M. (2006). Water and electrolyte needs for football training and match-play. *Journal of Sports Sciences*, 24 (7), 699-707.

Silva, R. P., Mündel, T., Natali, A. J., Bara-Filho, M. G., Lima, J. R. P., Alfenas, R. C. G., Lopes, P. R. N. R., Belfort, F. G. And Marins, J. C. B. (2011). Fluid balance of eleite Brazilian youth soccer players during consecutive days of training. *Journal of Sports Sciences*, 29 (7), 725-732.

Sokmen, B., Armstrong, L. E., Kraemer, W. J., Casa, D. J., Dias, J. C., Judelson, D.A. and Maresh, C. M. (2008). Caffeine use in sports: Considerations for the athlete. *Journal of Strength and Conditioning Research*, 22, 978-986.

Spriet, L. L. and Gibala, M. J. (2004). Nutritional strategies to influence adaptations to training. *Journal of Sports Sciences*, 22, 127-141.

Stolen, T., Chamari, K., Castagna,C. and Wilsløff, U. (2005). Physiology of soccer: An update. *Sports Medicine, 35(6),* 501-36.

Stone, K. J. and Oliver, J. L. (2009). The effect of 45 minutes of soccer-specific exercise on the performance of soccer skills. *International Journal of Sports Physiology and Performance*, 4, 163-175.

Tarnopolsky, M. A. (1994). Caffeine and endurance performance. *Sports Medicine*, 18 (2), 109-125.

Thatcher, R. and Batterham, A. M. (2004). Development and validation of a sport-specific exercise protocol for elite youth soccer players. *Journal of Sports Medicine and Physical Fitness*, 44 (1), 15-22.

Tsintzas, K., Liu, R., Campbell, I. and Gaitanos, G. (1993). The effect of carbohydrate ingestion on performance during a 30-km race. *International Journal of Sport Nutrition and Exercise Metabolism*, 3 (2), 127-139.

Van Gool, D., Van Gerven, D. and Boutmas, J. (1988). The physiological load imposed on soccer players during real match play. In T. Reilly, A. Leeds, K. Davids, and W. J. Murphy (Eds.), *Science and Football* (pp. 51-59). London: E & FN Spon.

Van Nieuwenhoven, M. A., Brummer, R. M. and Brouns, F. (2000). Gastrointestinal function during exercise: comparison of water, sports drink, and sports drink with caffeine. *Journal of Applied Physiology*, 89 (3), 1079-1085.

Vigne, G., Gaudino, C., Rogowski, I., Alloati, G. and Hautier, C. (2010). Activity profile in elite Italian championship team. *International Journal of Sports Medicine*, 31, 304-310.

Vist, G., Maughan, R. (1994). Gastric emptying of ingested solutions in man: effect of beverage glucose concentration. *Medicine and Science in Sports and Exercise*, 26 (10), 1269-1273.

Wagenmakers, A. J., Beckers, E. J., Brouns, F., Kuipers, H., Soeters, P. B., van der Vusse, G. J. and Saris, W. H. (1991). Carbohydrate supplementation, glycogen depletion, and amino acid metabolism during exercise. *American Journal of Physiology*, 260, E883-890.

Wagenmakers, A. J. M., Brouns, F., Saris, W. H. M. and Halliday, D. (1993). Oxidation rates of orally ingested carbohydrates during prolonged exercise in man. *Journal of Applied Physiology*, 75, 2774-2780.

Wallis, G. A., Rowlands, D. S., Shaw, C., Jentjens, R. L. and Jeukendrup, A. E. (2005). Oxidation of combined of maltodextrins and fructose during exercise. *Medicine and Science in Sports and Exercise*, 37 (3), 426-432.

Walsh, R., Noakes, T. D., Hawley, J. and Dennis, S. C. (1994). Impaired high-intensity cycling performance time at low levels of dehydration. *International Journal of Sports Medicine*, 15 (7), 392-398.

Weinberg, A. D. and Minaker, K. L. (1995). Dehydration. Evaluation and management in older adults. *Journal of the American Medical Association*, 274 (19), 1552-1556.

Welsh, R. S., Davis, J. M., Burke, J. R. and Williams, H. G. (2002). Carbohydrate and physical/mental performance during intermittent exercise to fatigue. *Medicine and Science in Sports and Exercise*, 34 (4), 723-731.

Weston, M., Batterham, A. M., Castagna, C., Portas, M. D., Barnes, C. A., Harley, J.A. and Lovell, R. J. (2011). Reduction in physical match performance at the start of the second half in elite soccer. *International Journal of Sports Physiology and Performance*, 6 (2), 174-182.

Widmaier, E. P., Raff, H. and Strang, K. T. (2011). The digestion and absorption of food. In E. P. Widmaier, H. Raff, and K. T. Strang (Eds), *Vander's Human Physiology: The mechanisms of body function* (pp. 516-553). New York: McGraw-Hill.

Williams, J. D., Abt, G. and Kilding, A. E. (2010). Ball-sport endurance and sprint test (BEAST90): validity and reliability of a 90-minute soccer performance test. *Journal of Strength and Conditioning*, 24 (12), 3209-3218.

Williams, C. and Serratosa, L. (2006). Nutrition on match day. *Journal of Sports Sciences*, 24 (7), 687-697.

Yeo, S. E., Jentjens, R. L., Wallis, G. A. and Jeukendrup, A. E. (2005). Caffeine increases exogenous carbohydrate oxidation during exercise. *Journal of Applied Physiology*, 99 (3), 844-850.

Zeederberg, C., Leach, L., Lambert, E. V., Noakes, T. D., Dennis, S. C. and Hawley, J. A. (1996). The effect of carbohydrate ingestion on the motor skill proficiency of soccer players. *International Journal of Sport Nutrition*, 6, 348-355.